

BIRLA CENTRAL LIBRARY

PILANI (RAJASTHAN)

Call No.

574.1926

V837

v.6

Accession No.

34723

VITAMINS AND HORMONES
VOLUME VI

VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

Edited by

ROBERT S. HARRIS
Professor of Biochemistry of Nutrition,
Massachusetts Institute of Technology,
Cambridge, Mass.

KENNETH V. THIMANN
Professor of Plant Physiology,
Harvard University,
Cambridge, Mass.

VOLUME VI

With Cumulative Subject Index of Vols. I-V



1948

ACADEMIC PRESS INC. PUBLISHERS
NEW YORK

Copyright 1948, by
ACADEMIC PRESS INC.
125 EAST 23RD STREET
NEW YORK-10, N. Y.

PRINTED IN THE UNITED STATES OF AMERICA

CONTRIBUTORS TO VOLUME VI

- HARRIET BONNER, *William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California*
- JAMES BONNER, *William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California*
- HENRIK DAM, *Danmarks Tekniske Højskole, København, Denmark*
- GLADYS A. EMERSON, *Merck Institute for Therapeutic Research, Rahway, New Jersey*
- BRIAN L. HUTCHINGS, *Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York*
- EDWARD C. KENDALL, *Division of Biochemistry, Mayo Foundation, Rochester, Minnesota*
- HANS MOLITOR, *Merck Institute for Therapeutic Research, Rahway, New Jersey*
- JOHN H. MOWAT, *Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York*
- B. S. SCHWEIGERT, *Division of Biochemistry and Nutrition, American Meat Institute Foundation, University of Chicago, Chicago, Illinois*
- H. M. SINCLAIR, *Wellcome Laboratory of Human Nutrition, University of Oxford*
- FRED W. TANNER, JR., *Chas. Pfizer and Co., Inc., Brooklyn, N. Y.*
- J. M. VAN LANEN, *Hiram Walker and Sons, Inc., and Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Illinois*

EDITORS' PREFACE

The favorable reception of preceding volumes makes it clear that *Vitamins and Hormones* continues to serve a valuable function. Indeed, both the wide scattering of the literature and its ever increasing volume are only too obvious to workers in the field. Some aspects of the breadth of this field are shown by three of the articles in this volume which bring out the importance of the vitamins as pharmacological agents, as plant hormones, and as products of industrial microbiology.

A cumulative subject index of Volumes I through V is included here. The compilation of this valuable index by Dr. Martha Sinai should make the series considerably more useful as a reference work.

It is regretted that unavoidable difficulties should have delayed the appearance of this volume by some four months. With the following volume it is expected that a return will be made to the normal schedule.

KENNETH V. THIMANN
ROBERT S. HARRIS

CONTENTS

	<i>Page</i>
CONTRIBUTORS TO VOLUME VI.....	v
EDITORS' PREFACE.....	vii

The Chemistry and Biological Action of Pteroylglutamic Acid and Related Compounds

BY BRIAN L. HUTCHINGS AND JOHN H. MOWAT, *Lederle Laboratories Division,
American Cyanamid Company, Pearl River, New York*

I. Introduction.....	1
II. Isolation of Vitamin B ₁₂	1
III. Isolation of Vitamin B ₁₂ Conjugate.....	2
IV. Isolation of the Liver <i>Lactobacillus casei</i> Factor.....	3
V. Isolation of the Fermentation <i>Lactobacillus casei</i> Factor.....	4
VI. Isolation of Rhizopterin (S.L.R. Factor).....	4
VII. Degradation of Vitamin B ₁₂	5
VIII. Degradation of Vitamin B ₁₂ Conjugate.....	7
IX. Degradation of the Fermentation <i>Lactobacillus casei</i> Factor.....	7
X. Degradation of Rhizopterin (S.L.R. Factor).....	11
XI. Synthesis of the Liver <i>Lactobacillus casei</i> Factor.....	13
XII. Synthesis of Pteroylpolyglutamic Acid Compounds.....	15
XIII. Antagonists of Pteroylglutamic Acid and Related Compounds.....	16
XIV. Biological Activity.....	18
XV. Vitamin B ₁₂ Conjugase (Pteroylglutamic Acid Conjugase).....	20
XVI. Quantitative Determination.....	21
XVII. Summary.....	22
References.....	22

Vitamin K

BY HENRIK DAM, *Danmarks Tekniske Højskole, København, Denmark*

I. Introduction.....	28
II. Vitamin K Active Compounds.....	28
III. Methods for the Determination of Vitamin K and Related Substances...	29
IV. Mode of Action of Vitamin K in Prothrombin Formation.....	33
V. Effects of Vitamin K and Related Substances on Processes Other than Prothrombin Formation.....	35
VI. Substances Causing Hypoprothrombinemia and Their Relationship to Vitamin K.....	38
VII. Application of Vitamin K in Human Medicine.....	43
VIII. Summary.....	47
References.....	48

Nutritional Requirements of the Cotton Rat and Hamster

By B. S. SCHWEIGERT, *Division of Biochemistry and Nutrition, American Meat Institute Foundation, University of Chicago, Chicago, Illinois*

	Page
I. Introduction.....	55
II. Nutritional Requirements of the Cotton Rat.....	56
III. Nutritional Requirements of the Hamster.....	61
IV. General Comments.....	64
V. Summary.....	65
References.....	66

Vitamins as Pharmacologic Agents

By HANS MOLITOR AND GLADYS A. EMERSON, *Merck Institute for Therapeutic Research, Rahway, New Jersey*

I. Introduction.....	69
II. Water Soluble Vitamins.....	75
III. Fat Soluble Vitamins.....	89
References.....	93

The Assessment of Human Nutriture

By H. M. SINCLAIR, *Wellcome Laboratory of Human Nutrition, University of Oxford*

I. Introduction.....	102
II. Dietary Assessment.....	114
III. Chemical Assessment.....	116
IV. Functional Assessment.....	121
V. Somatometric Assessment.....	129
VI. Clinical Assessment.....	131
VII. Discussion—Summary.....	150
References.....	157

Vitamins in Microorganisms—Distribution and Quantitative Synthesis

By J. M. VAN LANEN, *Hiram Walker and Sons, Inc., and Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Illinois*, AND FRED W. TANNER, JR., *Chas. Pfizer and Co., Inc., Brooklyn, N. Y.*

I. Introduction.....	164
II. Thiamine.....	166
III. Riboflavin.....	173
IV. Pantothenic Acid.....	181
V. Niacin.....	183
VI. Biotin.....	188
VII. <i>p</i> -Aminobenzoic Acid.....	191
VIII. Pyridoxine.....	194
IX. Inositol.....	196
X. Folic Acid.....	197
XI. Ascorbic Acid.....	202
XII. Ergosterol.....	204
XIII. Vitamin K.....	207
XIV. Carotenoids.....	208

CONTENTS

xi

	<i>Page</i>
XV. Vitamins in Fermentation Products.....	210
XVI. Summary.....	212
References.....	214

The B Vitamins as Plant Hormones

BY JAMES BONNER AND HARRIET BONNER, *William G. Kerckhoff Laboratories of the
Biological Sciences, California Institute of Technology, Pasadena, California*

I. Introduction.....	225
II. B Vitamins as Root Growth Factors.....	226
III. B Vitamins as Embryo Growth Factors.....	237
IV. Leaf Growth Factors.....	239
V. Stem Growth Factors.....	241
VI. Distribution of B Vitamins in the Plant.....	241
VII. Translocation of the B Vitamins.....	247
VIII. B Vitamins as Growth Factors for Intact Plants.....	254
IX. Vitamins of the B Complex as Growth Factors for Cuttings.....	259
X. Biochemistry and Metabolism of the B Vitamins.....	260
XI. Summary.....	268
References.....	270

The Influence of the Adrenal Cortex on the Metabolism of Water and Electrolytes

BY EDWARD C. KENDALL, *Division of Biochemistry, Mayo Foundation, Rochester,
Minnesota*

I. Introduction.....	278
II. Survival after Adrenalectomy.....	279
III. Normal Distribution of Electrolytes.....	281
IV. Abnormal Distribution of Electrolytes and Water.....	284
V. Renal Function.....	286
VI. Renal Function after Adrenalectomy.....	287
VII. The Transfer of Water and Electrolytes through the Intestinal Wall. . .	290
VIII. The Relation between Sodium Salts and the Hormones of the Adrenal Cortex.....	292
IX. The Influence of Sodium Chloride on Physiologic Processes.....	293
X. Symptoms of Adrenal Insufficiency.....	306
XI. Qualitative Differences of Steroids.....	312
XII. Electrolytes and Adrenal Insufficiency in Clinical Medicine.....	316
XIII. Comment and Summary.....	319
References.....	321
CUMULATIVE INDEX OF VOLUMES I-V.....	329
AUTHOR INDEX.....	391
SUBJECT INDEX.....	419

The Chemistry and Biological Action of Pteroylglutamic Acid and Related Compounds

By BRIAN L. HUTCHINGS AND JOHN H. MOWAT

Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

CONTENTS

	<i>Page</i>
I. Introduction.....	1
II. Isolation of Vitamin B ₆	1
III. Isolation of Vitamin B ₆ Conjugate.....	2
IV. Isolation of the Liver <i>Lactobacillus casei</i> Factor.....	3
V. Isolation of the Fermentation <i>Lactobacillus casei</i> Factor.....	4
VI. Isolation of Rhizopterin (S.L.R. Factor).....	4
VII. Degradation of Vitamin B ₆	5
VIII. Degradation of Vitamin B ₆ Conjugate.....	7
IX. Degradation of the Fermentation <i>Lactobacillus casei</i> Factor.....	7
X. Degradation of Rhizopterin (S.L.R. Factor).....	11
XI. Synthesis of the Liver <i>Lactobacillus casei</i> Factor.....	13
XII. Synthesis of Pteroylpolyglutamic Acid Compounds.....	15
XIII. Antagonists of Pteroylglutamic Acid and Related Compounds.....	16
XIV. Biological Activity.....	18
XV. Vitamin B ₆ Conjugase (Pteroylglutamic Acid Conjugase).....	20
XVI. Quantitative Determination.....	21
XVII. Summary.....	22
References.....	22

I. INTRODUCTION

In an excellent review Pffner and Hogan (1946) correlated the available pertinent data on what they termed the "Newer Hematopoetic Factors of the Vitamin B Complex." During the ensuing two years much of the information regarding the isolation, degradation and synthesis of the factors variously known as vitamin B₆, vitamin B₆ conjugate, liver *Lactobacillus casei* factor, fermentation *L. casei* factor and rhizopterin (S.L.R. factor) has been published and thus it is now possible to bring order to the confusing terminology and to correlate and present the data on a rational chemical basis. For the information prior to 1945, the reader is referred to the above mentioned review.

II. ISOLATION OF VITAMIN B₆

Pffner *et al.* (1943) reported the isolation of a compound which was active for *Lactobacillus casei* and which promoted growth and hemo-

globin formation in the chick. This factor was identical to that of Hogan and Parrott (1939, 1940) and was designated "vitamin B₆" because of its relation to chick nutrition. In a later publication (Piffner *et al.*, 1947) the isolation procedure and certain chemical properties of the compound were described.

After autolysis or suitable enzymatic digestion depending upon the tissue used as a source material, an aqueous extract was prepared. The active compound was thence successively adsorbed on and eluted from Amberlite IR-4, Super Filtrol, and Norite SG-11. The eluant in each case was either aqueous ammonia or a 5% ammonia-50% ethanol mixture. After removal of the ethanol and suitable concentration, the aqueous solution was extracted at pH 5.6 with butanol to remove non-acidic impurities. The active compound was then extracted into butanol at pH 3.0. After concentrating and cooling, the precipitated compound was redissolved in 90% methanol and then precipitated as the barium salt. The water soluble barium salts were treated with a solution of zinc acetate and the insoluble zinc salts collected. After decomposition of the zinc salt followed by reprecipitation as the barium salt, the free acid could be crystallized from hot water.

By this procedure or slight modifications thereof, crystals that exhibited identical properties were isolated from hog and horse liver and from yeast extract (Difco).

The pale yellow crystals exhibited no melting point but darkened and charred from 250°. The extremes of the analytical values were as follows:

C, 50.5-53.06; H, 4.05-5.00; N, 18.91-22.39 (Kjeldahl), 19.91-22.04 (Dumas).

Molecular weight determinations on the free acid gave values of 370 and 377 and on the dimethyl ester values of 463, 407, and 394 were obtained. Various salts and derivatives of the compound were prepared such as the diethyl ester, dimethyl ester dihydrochloride, diamide, silver salt, and disodium salt.

III. ISOLATION OF VITAMIN B₆ CONJUGATE

Yeast and yeast extracts are quite inactive when assayed microbiologically but are potent sources of antianemia activity when assayed by chicks. The isolation of the active compound present in such crude materials was announced by Piffner *et al.* (1945). The compound possessed approximately 0.4% activity for *L. casei* and 0.2% activity for *Streptococcus faecalis* R. The ultraviolet absorption spectra of the conjugate were similar to the spectra of vitamin B₆ with the difference

that the $E_{1\text{cm}}^{1\%}$ values were only 35.7% as great. The antianemic activity of the conjugate for the chick corresponded to the vitamin B₆ content of the compound as calculated from the ultraviolet absorption data. For further information the review of Pfiffner and Hogan (1946) should be consulted.

Only fragmentary data on the actual method of isolation of the conjugate has appeared. Pfiffner *et al.* (1947) have indicated that the preliminary isolation steps consisted of two consecutive adsorptions and elutions from Norite SG-11. No additional data have appeared.

IV. ISOLATION OF THE LIVER *Lactobacillus casei* FACTOR

Stokstad (1943) announced the isolation of a compound from liver and from yeast. In a later publication (Stokstad, Hutchings, and SubbaRow, 1946, 1948) the detailed procedure for the isolation of the liver compound was outlined. The source material was a dried 85% ethanol precipitate of an aqueous extract of liver. The active compound was adsorbed on and eluted from Norite A and Super Filtrol. This was followed by precipitation with barium and ethanol, and esterification of the precipitate, after drying, with methanolic hydrogen chloride. After esterification the solution was concentrated nearly to dryness and then dissolved in water and the active compound extracted into butanol. After removal of the butanol, the residue was dissolved in methanol and chromatographed on Super Filtrol. The column was developed with aqueous acetone and the 70% aqueous acetone eluates contained the activity. On removal of the acetone, the ester precipitated and was collected. Residual pigments were removed with small amounts of cold methanol. After reprecipitation from methanol, the purity of the compound could not be increased.

Combustion data for two samples were as follows:

Sample #1, C, 53.0; H, 4.6; N, 21.2

Sample #2, C, 53.1; H, 5.1; N, 20.5

After hydrolysis of the ester the free acid was crystallized from water. The extinction coefficients in 0.1 *N* sodium hydroxide at points of maxima were $E_{1\text{cm}}^{1\%}$: 255 μ , 565; 282 μ , 350; 365 μ , 195.

The compounds isolated by Pfiffner and coworkers from liver and yeast and the compound isolated by Stokstad and associates from liver were found to be essential for the growth of *Lactobacillus casei*, *Streptococcus faecalis* R. and the chick when compared under similar conditions. The ultraviolet absorption data and the analytical data were essentially in agreement and it was correctly deduced that the compounds were identical.

V. ISOLATION OF THE FERMENTATION *Lactobacillus casei* FACTOR

The isolation of a compound with different microbiological properties was reported by Hutchings *et al.* (1944). Later the source material was described and an outline of the method of isolation was presented (Hutchings, Stokstad, Bohonos, *et al.* (1946, 1948). The source material was a filtrate obtained from the aerobic growth of an organism classified as *Corynebacterium* sp. (Hutchings and Sloane, unpublished data). After removal of the bacterial cells the active compound was adsorbed on charcoal at pH 3.0 followed by elution with ethanolic ammonium hydroxide. The compound was then esterified, extracted into butanol, and fractionated from methanol. The purified ester was saponified with barium hydroxide. Residual extraneous pigments were removed by absorption with Florisil and precipitation of the compound as the free acid yielded essentially a pure product.

The compound could be crystallized only in the presence of electrolytes. If the electrolytes were absent, gels were invariably formed. Under the defined conditions it was possible to crystallize the compound as the free acid, barium salt, and methyl ester.

Consistent analytical data for the fermentation product were not obtained. The following values are representative:

C, 47.7—48.6; H, 4.4—4.8; N, 15.5—16.2

The fermentation compound is markedly more soluble as the free acid or as the methyl ester in various solvents than is the compound isolated from liver. The distinguishing characteristic of the compound is its relative activity for *Lactobacillus casei* and *Streptococcus faecalis* R. The fermentation compound is 60–80% as active as the liver compound for *L. casei* but only 4–6% as active for *S. faecalis* R.

Hutchings, Oleson, and Stokstad (1946) in a study of its activity for the chick reported data indicating that on a molar basis it was as active as the liver compound. The fermentation compound has also been reported as being efficacious in the treatment of vitamin M deficiency in the monkey (Day *et al.*, 1945) and in relieving the sulfonamide-induced deficiency in the rat (Daft and Sebrell, 1943).

VI. ISOLATION OF RHIZOPTERIN (S.L.R. FACTOR)

The isolation of a factor highly active for *Streptococcus lactis* R. but essentially inactive for *Lactobacillus casei* was reported by Keresztesy *et al.* (1943). Recently, the isolation, degradation, and synthesis of this compound has been described.

When natural materials are subjected to differential assay with *S. lactis* R. and *L. casei*, certain materials are much more highly active for *S. lactis* R. than for *L. casei*. The source material used by Rickes *et al.* (1947) exhibited this property to a marked degree. This material was a charcoal adsorbate derived from the purification of liquors of *Rhizopus nigricans* fumaric acid fermentation.

The crystalline compound was obtained by the following purification steps: elution of the charcoal adsorbate, readsorption on Norite A and elution, adsorption on Fullers earth and elution, precipitation at pH 7.0, chromatographic adsorption on alumina, and crystallization as the free acid or ammonium salt. The isolated compound was highly active for *S. lactis* R. (0.000034 γ /ml. for half-maximum growth), but inactive for *L. casei*, the chick and the rat.

Analytical data for the free acid suggested the formula $C_{15}H_{12}N_4O_4$. Potentiometric titration data indicated the substance was a dibasic acid with an equivalent weight calculated to be 167.5 or a molecular weight of 335. The compound was essentially insoluble in water and in the common organic solvents but dissolved readily in acid or alkali.

The ultraviolet absorption spectra of the compound had points of similarity to the spectra of xanthopterin. The ultraviolet absorption data and the physical properties of the compound suggested the presence of a pteridine nucleus. On this basis the name rhizopterin was suggested.

Wolf *et al.* (1947) in a refinement of the isolation technique used luteo ethylenediaminocobaltic chloride to precipitate rhizopterin from crude preparations. After crystallization from water, the compound was converted to rhizopterin by treatment with dilute acetic acid.

VII. DEGRADATION OF VITAMIN B₆

Wittle *et al.* (1947) reported the oxidative degradation of vitamin B₆. When the compound or its dimethyl ester was oxidized with either permanganate or chloric acid, a fluorescent microcrystalline solid was obtained. The compound contained a carboxyl group as evidenced by the formation of a methyl ester. Analytical values for the free acid varied. The possible empirical formulations were $C_8H_7O_3N_5$, $C_8H_6O_4N_6$, and $C_7H_5O_3N_5$. Further oxidation with chloric acid yielded guanidine. The formation of guanidine indicated the presence of a 2-aminopyrimidine ring. This evidence taken in conjunction with the analytical values and the absorption data strengthened the supposition that the oxidation product was a pterin.

A study of the ultraviolet absorption data of various pterins eliminated from consideration those having a hydroxyl group at position 6 or 7.

The compound that most nearly resembled the unknown acid was synthesized by condensing ethyl α,β -diketobutyrate with 2,4,5-triamino-6-hydroxypyrimidine. The structure assigned was that of 2-amino-4-hydroxy-6-carboxy-7-methylpteridine on the basis that the more reactive α -carbonyl in ethyl α,β -diketobutyrate condensed with the more basic 5-amino group in 2,4,5-triamino-6-hydroxypyrimidine. However, the ultraviolet absorption spectrum of the synthetic pteridine in 0.1 *N* acid was significantly different from that of the unknown compound. Furthermore, 2-amino-4-hydroxy-6-carboxy-7-methylpteridine on treatment with alkaline permanganate yielded the corresponding 6,7-dicarboxypteridine whereas the unknown acid was unchanged by this treatment. Subsequent disclosure by Angier *et al.* (1946) and Mowat, Boothe, *et al.* (1948) of their structural work indicated that the unknown acid was 2-amino-4-hydroxypteridine-6-carboxylic acid.

Ethyl acetate extraction of the chloric acid oxidized solution yielded a compound melting at 177–179°. Molecular weight determination and analytical values indicated that the compound had the formulation of $C_{12}H_{12}O_5N_2Cl_2$. A diester could be prepared, $C_{14}H_{16}O_5N_2Cl_2$, with either methanolic hydrogen chloride or diazomethane. If a sodium bromate-hydrobromic acid solution was used an analogous compound, $C_{12}H_{12}O_5N_2Br_2$, melting at 205–208° was obtained. When either of these compounds was heated at 210–240°, a decomposition took place and the products distilled from the melt. The compounds were separated by crystallization from dilute methanol. The soluble fraction, after crystallization from acetone, was identified by mixed melting points as *dl*-pyrrolidonecarboxylic acid.

The methanol insoluble fraction melted at 290–293° and from analysis the formula $C_7H_5O_2NCl_2$ was deduced. The disclosure of Angier *et al.* (1946) on the structure of pteroylglutamic acid indicated that the C_{12} dichloro compound should be 3,5-dichloro-4-aminobenzoylglutamic acid and the C_7 compound 3,5-dichloro-4-aminobenzoic acid. The identity of the two products was proved by comparison with synthetic samples of the above compounds.

Vitamin B₆ can be readily reduced in 0.1 *N* sodium hydroxide solution over platinum oxide or palladium to yield the dihydrovitamin (O'Dell *et al.*, 1947). During the reduction the characteristic yellow color of the compound is discharged. The ultraviolet absorption maxima in 0.1 *N* sodium hydroxide solution at 255 $m\mu$, 282 $m\mu$, and 365 $m\mu$ are destroyed and a new maximum at 284 $m\mu$ appears. If the reduction is carried out in glacial acetic acid over platinum, 2 moles of hydrogen are absorbed and the tetrahydro compound is formed. The ultraviolet absorption spectra of this compound in 0.1 *N* alkali are essentially similar to the

spectra of the dihydro derivative. Both reduced compounds on treatment with oxygen are oxidized to form the original compound.

Since O'Dell and coworkers could not readily hydrogenate leucopterin or isoxanthopterin using conditions similar to those for the reduction of vitamin B₆ to the dihydro derivative, and since xanthopterin readily adds 1 mole of hydrogen, it was postulated by them that the 7,8-double bond in vitamin B₆ (pteroylglutamic acid) is the readily reducible bond.

This ease of oxidation and reduction offers a possible role for pteroylglutamic acid in hydrogen transport in the oxidation-reduction enzyme systems.

VIII. DEGRADATION OF VITAMIN B₆ CONJUGATE

Ultraviolet absorption data of Piffner *et al.* (1945) indicated vitamin B₆ conjugate to consist of vitamin B₆ linked to an ultraviolet-transparent nitrogenous side chain. In later studies Piffner *et al.* (1946) reported that acid hydrolysis of the conjugate liberated 7 moles of glutamic acid. The conjugate is, therefore, vitamin B₆ plus 6 additional moles of glutamic acid.

IX. DEGRADATION OF THE FERMENTATION *Lactobacillus casei* FACTOR

The degradative studies leading to the elucidation of the structure of the liver *L. casei* factor and of the fermentation compound were carried out on the fermentation product.

Stokstad, Hutchings, Mowat, *et al.* (1946, 1948) described the degradation of the fermentation compound by anaerobic and aerobic alkaline hydrolysis and by acid hydrolysis.

Acid or alkaline hydrolysis liberated a fluorescent pigment and a diazotizable aromatic amine that could be estimated by the method of Bratton and Marshall (1939). The liberation of amine was most rapid in alkaline solution and oxygen was necessary for the cleavage. When the reaction was carried out anaerobically there was no change in the absorption spectrum and only small amounts of aromatic amine or fluorescent pigment were produced. However, two moles of α -amino acid nitrogen were liberated and because of its precipitability with barium and ethanol the formation of an aminodicarboxylic acid was suggested. The microbiological activity of the original compound had been significantly changed by the anaerobic hydrolysis. The ratio of activity for *L. casei* and *S. faecalis* R. was nearly unity. However, the compound resulting from alkaline hydrolysis was only 58% as active for *L. casei* and 57% as active for *S. faecalis* R. as was the compound isolated from liver. A racemic product was indicated and later, by comparison to a synthetic

product, the anaerobic alkaline hydrolysis product was shown to be the *dl*-liver compound.

The fluorescent pigment formed on aerobic alkaline hydrolysis analyzed for $C_7H_5N_5O_3$. The presence of a 2-aminopyrimidine group was suggested on the basis of the formation of guanidine on oxidation with chlorine water. The compound contained two acid groups with pKa values of 3.9 and 7.7. Decarboxylation of the compound yielded a new fluorescent monobasic acid with a pKa of 8.0. The decarboxylation data demonstrated the presence of a carboxylic and an enolic group in the fluorescent pigment derived from aerobic hydrolysis of the fermentation compound.

The empirical formula, ultraviolet absorption, titration data, and formation of guanidine suggested a 2-aminopteridine containing an enolic group and carboxyl group. Mowat, Boothe, *et al.* (1946, 1948) by synthesizing various pteridines showed the decarboxylated compound to be identical, on the basis of its ultraviolet absorption spectrum, with a synthetic pteridine of known structure, 2-amino-4-hydroxypteridine, prepared from 2,4,5-triamino-6-hydroxypyrimidine (Traube, 1900) and glyoxal.

These results suggested that the dibasic acid was probably a 2-amino-4-hydroxypteridine-6-(or 7) carboxylic acid. The truth of this assumption was demonstrated by the synthesis of the dibasic acid, first, by the chlorination and reduction of the previously known isoxanthopterin carboxylic acid (2-amino-4,7-dihydroxypteridine-6-carboxylic acid) (Purmann, 1941) whereby the 7-hydroxy group was replaced by hydrogen and second, by condensing 2,4,5-triamino-6-hydroxypyrimidine with ethyl- α -bromo- β , β -diethoxypropionate in the presence of silver carbonate. These syntheses established the nature of the ring system and the positions of the 2-amino group and the 4-hydroxy group. The location of the carboxyl group, although very probably in the 6-position, was not rigorously established, since the structures of xanthopterin and isoxanthopterin carboxylic acid postulated by Purmann (1940, 1941) were still open to question. Final proof that the carboxyl group of the dibasic acid was indeed in the 6-position was obtained from another pteridine degradation product of the fermentation *L. casei* factor.

Anaerobic acid hydrolysis (Stokstad, Hutchings, Mowat, *et al.*, 1946, 1948) of the fermentation compound yielded a fluorescent pigment and an aromatic amine. Butanol extraction at pH 7.0 of the hydrolyzate removed a pteridine monobasic acid. Crystallization of this compound from alkali produced an analytical product which by comparison to a synthetic pteridine (Mowat, Boothe, *et al.*, 1946, 1948) established its identity as 2-amino-4-hydroxy-6-methylpteridine. This latter compound

was synthesized by reacting 2,4,5-triamino-6-hydroxypyrimidine with methyl γ,γ -dimethoxyacetoacetate to give 2-amino-4-hydroxypteridine-6-acetic acid (which could be oxidized with hot alkaline potassium permanganate solution to 2-amino-4-hydroxypteridine-6-carboxylic acid). Decarboxylation of the pteridineacetic acid gave the desired 2-amino-4-hydroxy-6-methylpteridine identical with that obtained from the fermentation *L. casei* factor. Using a reaction analogous to that carried out by Weijlard *et al.* (1945) in their degradation of lumazines, the 6-methylpteridine was reacted with 4.2 *N* sodium hydroxide solution at 170°C. for 20 hours. The product, 2-amino-5-methylpyrazine-3-carboxylic acid, was isolated and decarboxylated to give 2-amino-5-methylpyrazine which was identical with a known sample prepared from 2,5-dimethylpyrazine. The formation of 2-amino-5-methylpyrazine provides conclusive proof that the pteridine degradation products of the *L. casei* factor(s) are substituted in the 6-position.

Since this review is primarily concerned with the chemical and biological characteristics of the *L. casei* factors, no attempt will be made to cover the field of pteridine chemistry *per se*. The reader who is interested in the earlier work in this field is referred to an excellent review of the chemistry of pteridines by Gates (1947) covering the published data up to 1947, as well as to a number of more recent publications (Elion and Hitchings, 1947; Cain *et al.*, 1946; Mallette *et al.*, 1947; Karrer *et al.*, 1947; Petering and Weisblat, 1947).

The aromatic amine fraction was obtained by hydrolyzing the fermentation compound anaerobically with alkali to yield the *dl*-liver *L. casei* factor (Stokstad, Hutchings, Mowat, *et al.*, 1946, 1948). This compound was then hydrolyzed aerobically with alkali and the resulting pteridines removed by precipitation with silver at pH 3.0. The aromatic amine was then precipitated with barium and alcohol to obtain a product analyzing for 2.1 atoms of total nitrogen for each atom of aromatic amine nitrogen. Subsequent hydrolysis with 2.0 *N* sulfuric acid liberated 45% of the nitrogen as α -amino acid nitrogen. From such hydrolyzates *p*-aminobenzoic acid was isolated and identified by mixed melting points and by its microbiological activity for *Clostridium acetobutylicum* and *Acetobacter suboxydans*.

Further degradative studies on the fermentation factor by Hutchings, Stokstad, Mowat, *et al.* (1946, 1948) made possible the probable structural formulation of the liver *L. casei* factor.

When the fermentation compound was autoclaved at pH 4.0 in aqueous solution, extensive biological inactivation occurred. Concentration of the clarified solution followed by solvent extraction and sublimation yielded a compound, m.p. 147–148°, with an empirical formula

of $C_6H_7O_3N$. Titration data were indicative of the presence of a carboxylic acid group and the equivalent weight was found to be 129.2. Alkaline hydrolysis converted the nitrogen into α -amino acid nitrogen. Microbiological assay of the hydrolyzates demonstrated the formation of glutamic acid. When the unknown was compared to an authentic sample of *l*-pyrrolidonecarboxylic acid, the two were found to be identical.

Sulfurous acid hydrolysis at room temperature of the fermentation compound gave rise to a pteridine and an aromatic amine fraction. The pteridine fraction reacted readily with typical aldehyde reagents. The analytical values for the phenylhydrazone suggested the presence of a pteridine carboxaldehyde.

If the freshly cleaved aldehyde was allowed to stand in dilute alkali anaerobically, the compound underwent a dismutation to yield approximately equivalent amounts of compounds identified as 2-amino-4-hydroxypteridine-6-carboxylic acid and 2-amino-4-hydroxy-6-methylpteridine. The former compound was identified by a comparison of its ultraviolet absorption spectrum with an authentic sample of 2-amino-4-hydroxypteridine-6-carboxylic acid. The methylpteridine was identified by analysis and comparison of its ultraviolet and infrared absorption spectra to those of a known sample of 2-amino-4-hydroxy-6-methylpteridine.

The aromatic amine fraction could not be crystallized but satisfactory analytical data were obtained on the barium salt. Of the nitrogen, 25% was present as aromatic amine nitrogen as measured by the method of Bratton and Marshall. The remaining nitrogen could by acid or alkaline hydrolysis be converted into α -amino acid nitrogen. Microbiological assay of the acid hydrolyzates gave analytical values commensurate with those calculated for 3 moles of glutamic acid. From alkaline hydrolyzates of the amine fraction, *p*-aminobenzoic acid was isolated and characterized by analysis and mixed melting points.

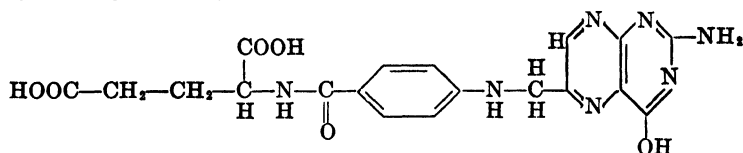
When the fermentation *L. casei* factor was hydrogenated over Raney nickel or palladium on barium sulfate at pH 7.0 and atmospheric pressure, no measurable biological inactivation occurred. However, if the hydrogenation with the palladium-barium sulfate catalyst was carried out at pH 1.0 or 3.0, there was rapid biological inactivation with the simultaneous formation of an aromatic amine. The pteridine moiety was reduced to a compound that exhibited markedly less ultraviolet absorption. An identical cleavage was effected by chemical means, i.e., zinc dust and hydrochloric or sulfuric acids. From such solutions, after reoxidation with manganese dioxide, a pteridine was isolated and characterized as 2-amino-4-hydroxy-6-methylpteridine.

The degradative data on the fermentation *L. casei* factor permitted the following conclusions. The formation of a primary aromatic amine

and a pteridine by aerobic alkaline hydrolysis, acid hydrolysis, sulfite cleavage, and chemical or catalytic reduction made it probable that the point of attachment was through the aromatic amine group to the pteridine. That the bridge at the point of attachment consisted of a single carbon atom was apparent from the formation of either 2-amino-4-hydroxypteridine-6-carboxylic acid or 2-amino-4-hydroxy-6-methylpteridine or a compound that could be converted into them as degradation products in several methods of cleavage. The evidence suggested that the carbon was present as a methylene group. The necessity for oxygen for the alkaline hydrolysis and the reductive formation of the methylpteridine were in accord with this hypothesis. This assumption was further strengthened by the observation that a simple model compound, *N*-benzyl-*p*-aminobenzoic acid (Mowat, Boothe, *et al.*, 1946, 1948), was stable in the presence of hot 1 *N* sodium hydroxide solution in the complete absence of oxygen, but was readily cleaved if oxygen was present, just as in the case of the fermentation *L. casei* factor.

The aromatic amine formed on cleavage with sulfurous acid was a tetrapeptide composed of 3 moles of glutamic acid and 1 mole of *p*-aminobenzoic acid. In degrading the fermentation compound to the *dl*-liver *L. casei* factor, 2 moles of glutamic acid were liberated. Hence, the liver *L. casei* factor contains 1 mole of glutamic acid and the fermentation compound, 3 moles of glutamic acid.

The liver *L. casei* factor is therefore *N*-[4-[(2-amino-4-hydroxy-6-pteridyl)methyl]amino}benzoyl]-*l*(+)-glutamic acid.



X. DEGRADATION OF RHIZOPTERIN (S.L.R. FACTOR)

When a solution of rhizopterine was allowed to stand in dilute alkali, the *S. lactis* R. activity of the solution decreased to one-tenth that of rhizopterine but the *L. casei* activity remained unchanged (Rickes, Trenner, *et al.*, 1947). From such solutions a compound analyzing for $C_{14}H_{10}N_6O_2Na_2$ was isolated. The new compound, termed aporhizopterine, was a weakly dibasic acid, having a calculated equivalent weight of 150.

The new compound exhibited different ultraviolet absorption characteristics than did rhizopterine. In dilute alkali the absorption increases in the region of 280 $m\mu$. In acid solution, the compound exhibits maxima at 250 $m\mu$ and 302.5 $m\mu$ whereas rhizopterine was characterized by absorp-

tion bands at 252.5 $m\mu$ and 322.5 $m\mu$. The spectra of aporhizopterin closely resembled the spectra for vitamin B₆ (pteroylglutamic acid), the only difference being in the intensity of the absorption bands. Keresztesy and coworkers concluded that aporhizopterin had the same chromophoric group as vitamin B₆ but differed from it in having a smaller mass.

Further degradative data by Wolf *et al.* (1947) led to a complete characterization of rhizopterin. Various derivatives of rhizopterin were prepared (acetyl, methoxyacetyl, phenylacetyl, benzoyl). A molecular weight determination on acetylrhizopterin gave a value of 369 or a molecular weight of 327 for the parent compound. Potentiometric titration of acetyl rhizopterin indicated the presence of a carboxyl group and a weakly acid group.

Acid hydrolysis of rhizopterin liberated formic acid which was characterized as the *p*-bromophenacyl ester. Formic acid was also formed on treatment of rhizopterin with alkali. After acid or alkaline hydrolysis, the product would form a diacetyl derivative.

When the alkaline hydrolysis product (aporhizopterin) was oxidized with potassium chlorate in the presence of excess hydrochloric acid, chloranil and oxaloguanidine were obtained. The former product indicated the presence of a substituted benzenoic nucleus whereas the latter product suggested the presence of a 2-aminopyrimidine ring.

Thermal decomposition of the alkaline hydrolysis product or rhizopterin yielded *p*-aminobenzoic acid characterized as *p*-acetamidobenzoic acid.

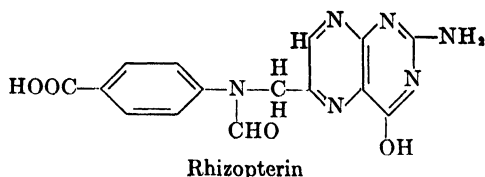
The position of the formyl group was deduced from the fact that oxidation of benzoyl xanthopterine or benzoylrhizopterine formed benzoylguanidine. This evidence when taken in conjunction with the fact that rhizopterine contains one acylatable group and aporhizopterine two acylatable groups locates the formyl group on the nitrogen of the *p*-aminobenzoic acid moiety.

Treatment of rhizopterine with sodium nitrite in concentrated hydrochloric acid solution removed the 2-amino group to form a compound, C₁₅H₁₁N₅O₅, designated as desiminorhizopterine. Acid hydrolysis of this product readily removed the formyl group.

When either desiminorhizopterine or aporhizopterine was treated with nitrous acid, a stable nitroso derivative was obtained demonstrating the secondary nature of the *p*-aminobenzoic acid nitrogen.

The formyl, 2-amino-4-hydroxypteridine and *p*-aminobenzoic acid groupings accounted for all of the elements in the molecular formula of rhizopterine. As the analyses for *C*-methyl, *O*-methyl and *N*-methyl were negative, the remaining carbon atom must be present as a bridge.

The proof of structure of rhizopterin was definitely established when the compound was synthesized by reacting formic acid with pterioic acid. Rhizopterin is therefore *p*-[*N*-(2-amino-4-hydroxypyrimido[4,5-*b*]pyrazin-6-ylmethyl)-formamido]benzoic acid.



XI. SYNTHESIS OF THE LIVER *Lactobacillus casei* FACTOR

Four different methods for the synthesis of the liver *L. casei* factor have been described.

The first, that of Waller *et al.* (1946, 1948) was effected by adding an alcoholic solution of 2,3-dibromopropionaldehyde to a stirred, aqueous solution of 2,4,5-triamino-6-hydroxypyrimidine and *p*-aminobenzoyl-*l*(+)-glutamic acid while maintaining the reaction mixture at about pH 4.0 by the addition of alkali or by the use of a suitable buffer. The precipitated crude product, containing approximately 15% by weight of biologically active material, was collected and purified.

Similar material has been synthesized by Hultquist *et al.* (1947, 1948) using the following stepwise procedure.

2,3-dibromopropionaldehyde was condensed with pyridine to give the quaternary salt, *N*-[2-formyl-2-bromoethyl]pyridinium bromide. Condensation of this substance with 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride in the presence of potassium iodide yielded the crystalline *N*-[(2-amino-4-hydroxy-6-pteridyl)methyl]pyridinium iodide. This compound was reacted with *p*-aminobenzoyl-*l*(+)-glutamic acid in hot ethylene glycol in the presence of sodium methoxide. The reaction mixture, after dilution with water and acidification to pH 3.0, gave a precipitate containing about 25% by weight of biologically active material.

A third method of synthesis, that of Angier *et al.* (1948) involved the condensation of diethyl *N*-(*p*-aminobenzoyl)-*l*(+)-glutamate with reductone (2,3-dihydroxyacrylaldehyde) to give diethyl *N*-{*p*-(2,3-dihydroxy-2-ene-propylideneamino)-benzoyl}glutamate. A mixture of this substance and 2,4,5-triamino-6-hydroxypyrimidine, when heated in ethylene glycol at 135° gave a crude product containing about 20% of active material.

In a fourth synthesis, that of Boothe, Waller, *et al.* (1948), 2-amino-4-

hydroxy-6-methylpteridine was treated with bromine in a sealed tube at 155° or with bromine in hot hydrobromic acid to yield a crude bromomethylpteridine. An analogous chloro compound was obtained when the methylpteridine was reacted with sulfuryl chloride in the presence of a trace of benzoyl peroxide.

Condensation of the crude bromomethylpteridine with diethyl *N*-(*p*-aminobenzoyl)-*l*(+)-glutamate in hot ethylene glycol yielded a crude product containing about 15% of biologically active material.

Condensation of the crude chloromethylpteridine by the same procedure gave a material containing about 1.0% biological activity.

Purification (Waller *et al.*, 1948) of the materials obtained from each of the four syntheses described above was effected by dissolving the crude reaction product in hot water at a concentration of 0.0002 g. of activity/ml. by the addition of sodium hydroxide to pH 10–12. This solution was adjusted to pH 7.0, cooled and filtered, and the active material was precipitated from the filtrate at pH 3.0. This precipitate was again dissolved in dilute alkali, clarified with charcoal, and reprecipitated at pH 3.0 by the addition of hot dilute acetic acid. This partially purified substance was collected and dissolved in dilute alkali at about 0.002 g./ml. and again clarified with charcoal. The solution was then diluted with hot water and acidified to pH 3.0 with acetic acid. On cooling the active compound crystallized. Further purification was effected by recrystallization from hot water.

Another purification procedure has also been described in an earlier publication (Waller *et al.*, 1946).

The purified products from each of the above syntheses were compared and found to be identical and to possess chemical, physical, and biological properties identical with those of the naturally occurring liver *L. casei* factor.

By the use of *p*-aminobenzoic acid instead of *p*-aminobenzoylglutamic acid in the above syntheses there was obtained [4-{(2-amino-4-hydroxy-6-pteridyl)methyl}-amino]-benzoic acid, the fundamental structural unit common to all of the known *L. casei* factors and rhizopterin. For obvious reasons, the chemical name of this compound is too cumbersome for general usage, and Waller *et al.* (1946, 1948) proposed for it the name "pteroic acid," and for the [4-{(2-amino-4-hydroxy-6-pteridyl)methyl}-amino]-benzoyl radical the name "pteroyl" was suggested. Using this nomenclature, the liver *L. casei* factor becomes pteroylglutamic acid, the fermentation *L. casei* factor becomes pteroyltriglutamic acid, etc. Of course, various modifications may be used to indicate which of several isomers is meant, as for example, pteroyl- γ -glutamyl- γ -glutamylglutamic acid, or pteroyl- α -glutamylglycine, etc.

XII. SYNTHESIS OF PTEROYLPOLYGLUTAMIC ACID COMPOUNDS

Hutchings, Stokstad, Mowat, *et al.* (1946, 1948) have stated that the so-called fermentation *L. casei* factor contains 3 molecules of glutamic acid in the peptide side chain. It is obvious that these three glutamic acid molecules could be arranged in five different but isomeric linkages. Which of these five possible structures of pteroyltriglutamic acid represented the fermentation *L. casei* factor could be determined either by a difficult stepwise degradation of the natural material or by chemical synthesis of these five possible forms, followed by direct comparison with the natural substance. Although the published record is not yet complete, a recent publication by Boothe, Mowat, *et al.* (1948) indicates that the fermentation *L. casei* factor is probably identical with synthetic pteroyl- γ -glutamyl- γ -glutamylglutamic acid. This substance was synthesized by the following procedure.

The γ -acid chloride of α -ethyl carbobenzoxyglutamate was condensed with diethylglutamate to give triethyl carbobenzoxy- γ -glutamylglutamate. After hydrogenolysis of the carbobenzoxy group, the dipeptide, triethyl- γ -glutamylglutamate hydrochloride, was isolated and again condensed with the γ -acid chloride of α -ethyl carbobenzoxyglutamate to give tetraethyl carbobenzoxy- γ -glutamyl- γ -glutamylglutamate. After hydrogenolysis of the carbobenzoxy group, the tripeptide was reacted with *p*-nitrobenzoyl chloride. The resulting *p*-nitrobenzoyl- γ -glutamyl- γ -glutamylglutamic acid tetraethyl ester was reduced to the corresponding *p*-aminobenzoyl- γ -glutamyl- γ -glutamylglutamic acid tetraethyl ester. This compound was then coupled with 2,4,5-triamino-6-hydroxypyrimidine and 2,3-dibromopropionaldehyde by a previously described method (Waller *et al.*, 1946, 1948).

The quantity of pteroyl- γ -glutamyl- γ -glutamylglutamic acid actually obtained was too small to permit complete purification, but biological assay data agreed with the values obtained from the natural fermentation *L. casei* factor.

In the same paper, Boothe and coworkers describe the synthesis and purification of one of the two possible isomers of pteroyldiglutamic acid, namely, pteroyl- γ -glutamylglutamic acid.

The other possible isomer of pteroyldiglutamic acid, pteroyl- α -glutamylglutamic acid, has been described by Mowat, Hutchings, *et al.* (1948). This compound was prepared by nitrobenzoylation and reduction of α -glutamylglutamic acid (Bergmann and Zervas, 1932) and condensation of the resulting *p*-aminobenzoyl- α -glutamylglutamic acid with 2,4,5-triamino-6-hydroxypyrimidine and 2,3-dibromopropionaldehyde. The purification of this substance is described.

Mowat and associates also describe the synthesis of another of the pteroyltriglutamic acid isomers, namely, pteroyl- α , γ -glutamyl diglutamic acid. This compound was prepared by condensing the γ -acid chloride of carbobenzoxy- α -glutamyl diethylglutamate with diethylglutamate to give the tetraethyl ester of carbobenzoxy- α , γ -glutamyl diglutamic acid. After removal of the carbobenzoxy group by hydrogenolysis, *p*-nitrobenzoylation, and reduction of the nitro compound, the resulting *p*-aminobenzoyl- α , γ -glutamyl diglutamic acid was condensed with 2,4,5-triamino-6-hydroxypyrimidine and 2,3-dibromopropionaldehyde to give assay quantities of the desired pteroyl- α , γ -glutamyl diglutamic acid. Biological assay data indicated that this isomer was not identical with the fermentation *L. casei* factor.

Although the synthesis of the three remaining isomers of pteroyltriglutamic acid has not yet been published, preliminary data available to the authors indicates that these isomers are probably not identical with the fermentation *L. casei* factor.

XIII. ANTAGONISTS OF PTEROYLGLUTAMIC ACID AND RELATED COMPOUNDS

It has often been noted that the effect of biologically active nutritional factors may be inhibited or even reversed by the administration of a so-called antimetabolite or antagonist.

During the past year a number of publications have appeared which describe substances antagonistic to the action of pteroylglutamic acid and related compounds.

Pteroylaspartic acid (Hutchings, Mowat, *et al.*, 1947) has been shown to be antagonistic to pteroylglutamic acid and its homologues in the nutrition of certain microorganisms, and of the chick. No inhibitory effect was observed in the rat. The "inhibition index" (the ratio of antagonist to metabolite required for one-half maximum inhibition) was found to be 2.0 for pteric acid, and 50.0 for pteroylglutamic acid, 7.0 for pteroyl- γ -glutamylglutamic acid, and 0.2 for pteroyl- γ -glutamyl- γ -glutamylglutamic acid in assays using *S. faecalis* R. as the test organism.

In the chick assay, about 500 parts of pteroylaspartic acid was found to inhibit the effect of 1.0 part of pteroylglutamic acid completely.

In the *L. casei* assay, the pteroylaspartic acid:pteroylglutamic acid inhibition index was about 1600, while in the *Escherichia coli* assay no inhibition was observed.

Another *L. casei* factor antagonist has been described by Martin, Tolman, and Moss (1947) and by Franklin *et al.* (1947). The compound was prepared by substantially the procedure of Waller *et al.* (1946, 1948) using 2,3-dibromobutyraldehyde instead of 2,3-dibromopropionaldehyde.

Martin and coworkers have called the active compound "7-methyl-folic acid," but so far no data have been presented to substantiate this structure.

Franklin and associates, using the crude unpurified material as an antagonist for pteroylglutamic acid found "inhibition ratios" of 20:1 for *S. faecalis* R., 1000:1 for *L. casei*, and 3000:1 for the rat.

The effect of the antagonist could be reversed by increasing the concentration of pteroylglutamic acid.

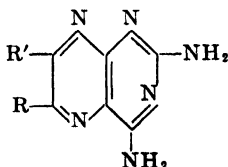
In rats, the pteroylglutamic acid deficiency syndrome was similar to but much more severe than that produced by succinylsulfathiazole, and oral lesions were observed that recall the oral lesions of monkeys deficient in Vitamin M (Day *et al.*, 1936).

Martin's group using their so-called "7-methyl *d*(-) folic acid" as an inhibitor for pteroylglutamic acid in the *S. faecalis* R. assay, reported an inhibition index of about 150, while in a later communication (Martin, Avakian, Tolman, *et al.*, 1947) they assign to 7-methylpteroyl-*l*(+)glutamic acid an inhibition index of 2. These workers also give inhibition indices for a number of other compounds: pteroylaspartic acid, 100; "7-methylpteroylaspartic acid," 100; "7-methylpteronic acid," 10; oxypteronic acid, inactive; oxypteroylglutamic acid, inactive; *N*-(4-[(4-quinazoline)amino] benzoyl)glutamic acid, inactive. This latter compound (Martin, Moss, and Avakian, 1947) showed some "folic acid" activity.

Still another pteroylglutamic acid inhibitor has been recently described by Seeger *et al.* (1947), namely *N*-[4-[(2,4-diamino-6-pteridyl)-methyl]-amino]-benzoyl]-glutamic acid. Using this pure material in the *S. faecalis* R. assay, the inhibition index was found to lie in the range of 1.9 to 0.4, depending upon the concentration of metabolite.

This pronounced inhibition of pteroylglutamic acid by its 4-amino analogue appears to be a function of the 4-amino group, since Daniel *et al.* (1947) have recently described experiments in which pteroylglutamic acid was inhibited by number of 4-aminopteridines, whereas the corresponding 2-amino-4-hydroxy, 2,4-dihydroxy, and 2-mercapto-4-hydroxy compounds showed little or no inhibitory effects.

The pteridines tested by Daniel and coworkers were prepared by Mallette *et al.* (1947) and by Cain *et al.* (1946). The inhibitory 4-amino-compounds had the following general structure.



The original papers should be consulted for a complete list of the pterins used in these experiments, but of all the compounds tested, 2,4-diamino-6,7-diphenylpyrimido(4,5-*b*)pyrazine and 2,4-diaminophenanthro(9,10-*e*)pyrimido(4,5-*b*)pyrazine were the most active inhibitors, using pteroylglutamic acid as the metabolite and *S. faecalis* R., *L. casei*, and *L. arabinosus* as the test organisms.

It is interesting to note that while both *S. faecalis* R. and *L. casei* require preformed pteroylglutamic acid and *L. arabinosus* is capable of synthesizing its own requirements of this substance yet competitive inhibition was observed with all three microorganisms.

It was also observed in the *L. arabinosus* assay, that 2 to 10 times as much pterin was required for growth inhibition if PABA was present.

In a later communication, Daniel *et al.* (1947) show that the growth of two other microorganisms which synthesize pteroylglutamic acid, *E. coli* and *Staphylococcus aureus*, is inhibited by these 2,4-diaminopterins. Furthermore a marked synergism was observed when low levels of the pterin and sulfathiazole were added to the culture medium.

The addition of pteroylglutamic acid completely overcame the growth inhibition caused by low levels of pterin and sulfathiazole and the effect of the compounds appeared to be competitive. If, however, the growth inhibitory effects of the pterin and the sulfathiazole were studied separately, it was found that the effect of adding pteroylglutamic acid was noncompetitive in nature.

Lampen and Jones (1946) have suggested that sulfonamides are antagonistic to pteroylglutamic acid by interfering with the biological synthesis of the pteroylglutamic acid, and Daniel *et al.* (1947) now suggest that the antagonistic effect of the 2,4-diaminopterins may be caused by interference with the use of pteroylglutamic acid and the above mentioned synergism is cited as evidence in favor of this hypothesis.

It seems desirable to point out that pure compounds should be used when studying the antagonistic or growth promoting action of these substances. In the preparation of pteroylglutamic acid and related compounds, the crude, unpurified reaction mixture may and in some cases has been shown to contain by-products with inhibitory properties. There is also evidence that two or more inhibitors having different biological action may occur in the same crude preparation.

XIV. BIOLOGICAL ACTIVITY

Pteric acid, rhizopterin, and the various polyglutamic acid analogues of pteroylglutamic acid have varying activities for *Lactobacillus casei*, *Streptococcus faecalis* R., and the chick. If the biological activity of

pteroylglutamic acid is taken as 100, the various compounds exhibit the activities noted in Table I.

TABLE I
Biological Activities of Certain Pteroyl Compounds

Compound	Activity for			Reference
	<i>L. casei</i> %	<i>S. faecalis</i> R. %	Chick %	
Pterioic acid.....	No	Yes ¹	No	Angier <i>et al.</i> (1946)
Rhizopterlin.....	No	10 × Molar activity	No	Rickes, Trenner, <i>et al.</i> (1947)
Pteroylglutamic acid.....	100	100	100	Angier <i>et al.</i> (1946)
Pteroyl- α -glutamylglutamic acid	0.5-1.0	0.5-1.0	Yes	Mowat, Hutchings, <i>et al.</i> (1948) Oleson, unpublished data
Pteroyl- α,γ -glutamylglutamic acid	0.5-1.0	0.5-1.0	Yes	Mowat, Hutchings, <i>et al.</i> (1948) Oleson, unpublished data
Pteroyl- γ -glutamylglutamic acid	Yes	Yes	Yes	Boothe, Mowat, <i>et al.</i> (1948) Oleson, unpublished data
Pteroyl- γ -glutamyl- γ -glutamylglutamic acid	Yes	2.0-4.0	Yes	Boothe, Mowat, <i>et al.</i> (1948) Oleson, unpublished data
Pteroylhexaglutamylglutamic acid.....	0.4	0.2	Yes	Pfiffner <i>et al.</i> (1945)

¹ Yes indicates the compounds are equimolecularly active.

Pterioic acid and rhizopterlin are active for *S. faecalis* R. but not for *L. casei* or the chick. Almost the reverse is true when the biological potencies of pteroyl- γ -glutamyl- γ -glutamylglutamic acid are evaluated.

The two compounds with alpha linked peptides, pteroyl- α -glutamylglutamic acid and pteroyl- α,γ -glutamylglutamic acid, are essentially inactive microbiologically but are fully active for the chick. Whether all analogues containing alpha linked peptides will be microbiologically inactive is impossible to state at this time.

Vitamin B₆ conjugate of Pfiffner *et al.* (1945) which has been designated as pteroylhexaglutamylglutamic acid (Pfiffner *et al.*, 1946) is inactive microbiologically but shows activity for the chick commensurate with its content of pteroylglutamic acid. After digestion with an enzyme

preparation from hog kidney or chicken pancreas, the hydrolyzed mixture has microbiological activities similar to those of pteroylglutamic acid.

The parallel microbiological inactivity of the conjugate and of the pteroyl compounds containing alpha linked peptides should not be construed as evidence that the conjugate has an α -peptide structure. There is no data in the literature indicating the configuration of the side chain of pteroylhexaglutamylglutamic acid.

From the microbiological and chick activities of the various compounds noted in Table I, it is most highly probable that the fractions designated variously as Factor U (Stokstad and Manning, 1938), Factor R (Schumacher *et al.*, 1940) and vitamins B₁₀ and B₁₁ (Briggs *et al.*, 1943) are, in fact, merely concentrates containing varying proportions of the above mentioned compounds. The fact that chicks can be raised on a diet containing known essentials is support for this view.

Scott *et al.* (1945) advanced the hypothesis that the lactone of either 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine or 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine facilitated the conversion of pteroyl- γ -glutamyl- γ -glutamylglutamic acid to pteroylglutamic acid which was then utilized by the chick. Hutchings, Oleson, and Stokstad (1946) under conditions of their experiments could not substantiate this finding. Jukes and Stokstad (1947) using diets similar to those of Scott and associates could show no beneficial effects on the addition of the lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine. The pteroyl- γ -glutamyl- γ -glutamylglutamic acid was as effective on a molar basis as was pteroylglutamic acid. It would seem, therefore, that the chick without dietary supplements can readily utilize the various conjugated forms of the vitamin.

XV. VITAMIN B₉ CONJUGASE (PTEROYLGLUTAMIC ACID CONJUGASE)

The enzyme from hog kidney that is responsible for the degradation of pteroylhexaglutamylglutamic acid with the liberation of pteroylglutamic acid has been designated by Pffner *et al.*, (1946) as a carboxypeptidase. From the behavior of the enzyme obtained from various sources it appears that at least two distinct conjugases occur in nature. Bird *et al.* (1946) in a study of the distribution of the conjugate-splitting enzyme found that the pH optimum of the conjugase from animal organs was approximately pH 4.5 whereas the enzyme derived from turkey pancreas had a pH optimum of 7.0-7.5. Prior to this the enzyme present in chicken pancreas had been described as having a pH optimum of 7.0 (Laskowski *et al.*, 1945).

From the studies of Pffner and associates it was apparent that most natural materials contain a substance(s) that is inhibitory to the act^{ion}

of the conjugase. In a continuance of this work Mims *et al.* (1947) demonstrated that nucleic acid either of the ribose or desoxyribose type was a strong *in vitro* inhibitor of the conjugase derived from hog kidney. Depolymerization of the nucleic acid by ribonuclease or thymonucleo-depolymerase, respectively, or treatment with such reducing agents as cysteine, ascorbic acid, or hydrogen sulfide destroyed the inhibiting property of the nucleic acid. The pteroylglutamic acid conjugase from chicken pancreas was not inhibited by nucleic acid and the data was offered as further proof of the existence of at least two distinct conjugase enzymes.

Fragmentary data was presented by Mims and coworkers indicating that nucleic acid had an *in vivo* action by depressing the expected excretion of pteroylglutamic acid when normal subjects were fed specified amounts of pteroylhexaglutamylglutamic acid.

An inhibition of the action of the pteroylglutamic acid conjugase from chicken pancreas and rat liver by the glutamic acid polypeptide of *p*-aminobenzoic acid (Ratner *et al.*, 1946) was reported by Sims and Totter (1947). The structural similarity of this compound and the *p*-aminobenzoic acid glutamic acid peptide moiety of pteroylhexaglutamylglutamic acid is obvious.

XVI. QUANTITATIVE DETERMINATION

The microbiological assay of pteroylglutamic acid has been adequately reviewed elsewhere (Stokstad and Hutchings, 1947).

For the chemical determination of the pteroyl compounds, Hutchings, Stokstad, Boothe, *et al.* (1947) have advanced a method based on the reductive cleavage of this group of compounds into their pteridin and aromatic amine components. The difference between the aromatic amine before and after reduction as determined by the method of Bratton and Marshall (1939) is used as a measure of the pteroyl derivative present.

The reduction is satisfactorily carried out in 0.5 *N* hydrochloric acid with zinc dust. A small amount of gelatin (0.005%) must be included in the reducing menstruum to inhibit the reductive destruction of the liberated amine. Under these conditions the reduction of pteroylglutamic acid proceeded essentially to completion.

The method is satisfactory for the determination of pteroylglutamic acid, its analogues and derivatives and for concentrates from natural sources that contain the active compound in excess of 5%.

The compounds in natural materials, both inorganic and organic, that may interfere with the determination have not been assessed. Additional work should be forthcoming along these lines.

XVII. SUMMARY

Degradation and synthesis have established the structure of the naturally occurring compounds variously designated as Vitamin B₆, liver *L. casei* factor, the fermentation *L. casei* factor, and rhizopterin. All of these compounds have a similar nucleus consisting of 4-[(2-amino-4-hydroxy-6-pteridyl)methyl] aminobenzoic acid. This compound or its formyl derivative (rhizopterin) exhibits activity for *S. faecalis* R., but is inactive for *L. casei*, the chick, and the rat. Pteroylglutamic acid, *N*-[4-[(2-amino-4-hydroxy-6-pteridyl)methyl]-amino]-benzoyl]-glutamic acid, (Vitamin B₆) (liver *L. casei* factor) is the simplest structure that shows both microbiological and animal activity. Extension of the glutamic acid side chain in either the α - or γ -position alters the microbiological activity, but the compounds retain equimolecular activity for the chick.

Certain compounds structurally related to pteroylglutamic acid have been prepared and shown to have antipteroylglutamic acid activity.

REFERENCES

- Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., Waller, C. W., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1946. *Science* **103**, 667-669.
- Angier, R. B., Stokstad, E. L. R., Mowat, J. H., Hutchings, B. L., Boothe, J. H., Waller, C. W., Semb, J., SubbaRow, Y., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1948. *J. Am. Chem. Soc.* **70**, 25-26.
- Bergmann, M., and Zervas, L. 1932. *Ber.* **65**, 1192.
- Bird, O. D., Robbins, M., Vandenbelt, J. M., and Piffner, J. J. 1946. *J. Biol. Chem.* **163**, 649-659.
- Boothe, J. H., Waller, C. W., Stokstad, E. L. R., Hutchings, B. L., Mowat, J. H., Angier, R. B., Semb, J., SubbaRow, Y., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1948. *J. Am. Chem. Soc.* **70**, 27-28.
- Boothe, J. H., Mowat, J. H., Hutchings, B. L., Angier, R. B., Waller, C. W., Stokstad, E. L. R., Semb, J., Gazzola, A. L., and SubbaRow, Y. 1948. *J. Am. Chem. Soc.* **70**, 1099-1102.
- Bratton, A. C., and Marshall, E. K., Jr. 1939. *J. Biol. Chem.* **128**, 537-550.
- Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B. 1943. *J. Biol. Chem.* **148**, 163-172.
- Cain, C. K., Mallette, M. F., and Taylor, E. C., Jr. 1946. *J. Am. Chem. Soc.* **68**, 1996-1999.
- Daft, F. S., and Sebrell, W. H. 1943. *U.S. Pub. Health Repts.* **58**, 1542-1545.
- Daniel, L. J., Norris, L. C., Scott, M. L., and Heuser, G. F. 1947. *J. Biol. Chem.* **169**, 689-697.
- Daniel, L. J., and Norris, L. C. 1947. *J. Biol. Chem.* **170**, 747-756.

- Day, P. L., Langston, W. C., and Shukers, C. F. 1936. *J. Biol. Chem.* **114**, xxv.
- Day, P. L., Mims, V., Totter, J. R., Stokstad, E. L. R., Hutchings, B. L., and Sloane, N. H. 1945. *J. Biol. Chem.* **157**, 423-424.
- Elion, G. B., and Hitchings, G. H. 1947. *J. Am. Chem. Soc.* **69**, 2553-2555.
- Franklin, A. L., Stokstad, E. L. R., Belt, M., and Jukes, T. H. 1947. *J. Biol. Chem.* **169**, 427-435.
- Gates, M. 1947. *Chem. Rev.* **41**, 63-95.
- Hogan, A. G., and Parrott, E. M. 1940. *J. Biol. Chem.* **132**, 507-517; 1939. *ibid.* **128**, xlv.
- Hultquist, M. E., Kuh, E., Cosulich, D. B., Fahrenbach, M. J., Northey, E. H., Seeger, D. R., Sickels, J. P., Smith, J. M., Jr., Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., and Waller, C. W. 1947. *N. Y. Acad. Sci.* **48**, (Supplement) I-VI.
- Hultquist, M. E., Kuh, E., Cosulich, D. B., Fahrenbach, M. J., Northey, E. H., Seeger, D. R., Sickels, J. P., Smith, J. M., Jr., Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., and Waller, C. W. 1948. *J. Am. Chem. Soc.* **70**, 23-24.
- Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., and Slobodkin, N. H. 1944. *Science* **99**, 371.
- Hutchings, B. L., Oleson, J. J., and Stokstad, E. L. R. 1946. *J. Biol. Chem.* **163**, 447-453.
- Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., Sloane, N. H., and SubbaRow, Y. 1946. *Ann. N. Y. Acad. Sci.* **48**, 265-267.
- Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., Sloane, N. H., and SubbaRow, Y. 1948. *J. Am. Chem. Soc.* **70**, 1-3.
- Hutchings, B. L., Stokstad, E. L. R., Mowat, J. H., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y. 1946. *Ann. N. Y. Acad. Sci.* **48**, 273-277.
- Hutchings, B. L., Stokstad, E. L. R., Mowat, J. H., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y. 1948. *J. Am. Chem. Soc.* **70**, 10-13.
- Hutchings, B. L., Stokstad, E. L. R., Boothe, J. H., Mowat, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y. 1947. *J. Biol. Chem.* **163**, 705-710.
- Hutchings, B. L., Mowat, J. H., Oleson, J. J., Stokstad, E. L. R., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y. 1947. *J. Biol. Chem.* **170**, 323-328.
- Hutchings, B. L., and Sloane, N. H. Unpublished data.
- Jukes, T. H., and Stokstad, E. L. R. 1947. *J. Biol. Chem.* **168**, 563-567.
- Karrer, P., Schwyzer, R., Erden, B., and Siegwart, A. 1947. *Helv. Chim. Acta* **30**, 1031-1036.
- Keresztesy, J. C., Rickes, E. L., and Stokes, J. L. 1943. *Science* **97**, 465.
- Lampen, J. O., and Jones, M. J. 1946. *J. Biol. Chem.* **166**, 435-448.
- Laskowski, M., Mims, V., and Day, P. L. 1945. *J. Biol. Chem.* **157**, 731-739.
- Mallette, M. F., Taylor, E. C., Jr., and Cain, C. K. 1947. *J. Am. Chem. Soc.* **69**, 1814-1816.
- Martin, G. J., Tolman, L., and Moss, J. 1947. *Arch. Biochem.* **12**, 318-319.
- Martin, G. J., Moss, J., and Avakian, S. 1947. *J. Biol. Chem.* **167**, 737.
- Martin, G. J., Avakian, S., Tolman, L., Urist, H., and Moss, J. Abstracts of Papers, Am. Chem. Soc. Meeting, Sept. 1947.
- Mims, V., Swendseid, M. E., and Bird, O. D. 1947. *J. Biol. Chem.* **170**, 367-377.
- Mowat, J. H., Boothe, J. H., Hutchings, B. L., Stokstad, E. L. R., Waller, C. W.,

- Angier, R. B., Semb, J., Cosulich, D. B., and SubbaRow, Y. 1946. *Ann. N. Y. Acad. Sci.* **48**, 279-281.
- Mowat, J. H., Boothe, J. H., Hutchings, B. L., Stokstad, E. L. R., Waller, C. W., Angier, R. B., Semb, J., Cosulich, D. B., and SubbaRow, Y. 1948. *J. Am. Chem. Soc.* **70**, 14-18.
- Mowat, J. H., Hutchings, B. L., Angier, R. B., Stokstad, E. L. R., Boothe, J. H., Waller, C. W., Semb, J., and SubbaRow, Y. 1948. *J. Am. Chem. Soc.* **70**, 1096-1098.
- O'Dell, B. L., Vandenbelt, J. M., Bloom, E. S., and Piffner, J. J. 1947. *J. Am. Chem. Soc.* **69**, 250-253.
- Oleson, J. J. Unpublished data.
- Petering, H. G., and Weisblat, D. I. 1947. *J. Am. Chem. Soc.* **69**, 2566-2567.
- Piffner, J. J., Binkley, S. B., Bloom, E. S., Brown, R. A., Bird, O. D., Emmett, A. D., Hogan, A. G., and O'Dell, B. L. 1943. *Science* **97**, 404-405.
- Piffner, J. J., Calkins, D. G., O'Dell, B. L., Bloom, E. S., Brown, R. A., Campbell, C. J., and Bird, O. D. 1945. *Science* **102**, 228-230.
- Piffner, J. J., Calkins, D. G., Bloom, E. S., and O'Dell, B. L. 1946. *J. Am. Chem. Soc.* **68**, 1392.
- Piffner, J. J., and Hogan, A. G. 1946. *Vitamins and Hormones* **4**, 1-31.
- Piffner, J. J., Binkley, S. B., Bloom, E. S., and O'Dell, B. L. 1947. *J. Am. Chem. Soc.* **69**, 1476-1487.
- Purmann, R. 1940. *Ann.* **546**, 98-102.
- Purmann, R. 1941. *Ann.* **548**, 284-292.
- Ratner, S., Blanchard, M., and Green, D. E. 1946. *J. Biol. Chem.* **164**, 691-701.
- Rickes, E. L., Chaiet, L., and Keresztesy, J. C. 1947. *J. Am. Chem. Soc.* **69**, 2749-2751.
- Rickes, E. L., Trenner, N. R., Conn, J. B., and Keresztesy, J. C. 1947. *J. Am. Chem. Soc.* **69**, 2751-2753.
- Schumacher, A. E., Heuser, G. F., and Norris, L. C. 1940. *J. Biol. Chem.* **135**, 313-320.
- Scott, M. L., Norris, L. C., Heuser, G. F., and Bruce, W. F. 1945. *J. Biol. Chem.* **158**, 291-298.
- Seeger, D. R., Smith, J. M., Jr., and Hultquist, M. E. 1947. *J. Am. Chem. Soc.* **69**, 2567.
- Sims, E. S., and Totter, J. R. 1947. *Federation Proc.* **6**, 291.
- Stokstad, E. L. R., and Manning, P. D. V. 1938. *J. Biol. Chem.* **125**, 687-696.
- Stokstad, E. L. R. 1943. *J. Biol. Chem.* **149**, 573-574.
- Stokstad, E. L. R., and Hutchings, B. L. 1947. *Biol. Symposia* **12**, 339.
- Stokstad, E. L. R., Hutchings, B. L., and SubbaRow, Y. 1946. *Ann. N. Y. Acad. Sci.* **48**, 261-263.
- Stokstad, E. L. R., Hutchings, B. L., and SubbaRow, Y. 1948. *J. Am. Chem. Soc.* **70**, 3-5.
- Stokstad, E. L. R., Hutchings, B. L., Mowat, J. H., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y. 1946. *Ann. N. Y. Acad. Sci.* **48**, 269-273.
- Stokstad, E. L. R., Hutchings, B. L., Mowat, J. H., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y. 1948. *J. Am. Chem. Soc.* **70**, 5-9.
- Traube, W. 1900. *Ber.* **33**, 1371-1383.
- Waller, C. W., Hutchings, B. L., Mowat, J. H., Stokstad, E. L. R., Boothe, J. H., Angier, R. B., Semb, J., SubbaRow, Y., Cosulich, D. B., Fahrenbach, M. J.,

- Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1946. *Ann. N. Y. Acad. Sci.* **48**, 283-286.
- Waller, C. W., Hutchings, B. L., Moyat, J. H., Stokstad, E. L. R., Boothe, J. H., Angier, R. B., Semb, J., SubbaRow, Y., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1948. *J. Am. Chem. Soc.* **70**, 19-22.
- Weijlard, J., Tishler, M., and Erickson, A. E. 1945. *J. Am. Chem. Soc.* **67**, 802-806.
- Wittle, E. L., O'Dell, B. L., Vandenbelt, J. M., and Pfiffner, J. J. 1947. *J. Am. Chem. Soc.* **69**, 1786-1792.
- Wolf, D. E., Anderson, R. C., Kaczka, E. A., Harris, S. A., Arth, G. E., Southwick, P. L., Mozingo, R., and Folkers, K. 1947. *J. Am. Chem. Soc.* **69**, 2753-2759.

Vitamin K

By HENRIK DAM

Danmarks Tekniske Højskole, København, Danmark

CONTENTS

	<i>Page</i>
I. Introduction.....	28
II. Vitamin K Active Compounds.....	28
III. Methods for the Determination of Vitamin K and Related Substances...	29
1. Blood Clotting Tests.....	29
2. Chemical Tests.....	29
A. Colorimetric Methods.....	29
Reactions with Dinitrophenylhydrazine.....	29
Craven's Reaction (1931) with Ethyl Cyanoacetate.....	30
The Reactions with Sodium Diethyl Dithiocarbamate.....	30
Reactions with Sodium Ethylate.....	30
Other Color Reactions.....	31
B. Redox Methods.....	32
C. Colorimetric Redox Methods.....	32
D. Polarography.....	33
E. Fluorometry.....	33
F. Spectroscopic Method.....	33
IV. Mode of Action of Vitamin K in Prothrombin Formation.....	33
V. Effects of Vitamin K and Related Substances on Processes Other than Prothrombin Formation.....	35
1. Possible Influence on Bleeding Tendency in Other Ways than by Prothrombin Formation.....	35
2. Postulated Influence on Blood Pressure.....	35
3. Postulated Effect on Proteolytic Enzymes.....	35
4. Postulated Lipotropic Effect.....	35
5. Influence on Metabolic Processes and Growth of Heterotrophic Organisms.....	36
6. Influence on Photosynthesis Respiration etc. in Photosynthesizing Microorganisms.....	37
7. Effect on Mitosis.....	38
VI. Substances Causing Hypoprothrombinemia and Their Relation to Vitamin K.....	38
1. Dicumarol.....	38
2. Isosteres of Vitamin K with Antagonistic Action.....	40
3. Salicylates.....	41
4. Dihydroxystearic Acid.....	41
5. Vitamin A in Massive Doses.....	41
6. Mineral Oil.....	42

	<i>Page</i>
7. Sulfa Drugs.....	42
8. Other Compounds.....	42
VII. Application of Vitamin K in Human Medicine.....	43
1. The Well Established Therapeutic Applications of Vitamin K in Cases with Generally Recognized Hypoprothrombinemia.....	43
2. Hypoprothrombinemia of Pancreatic Origin.....	44
3. Diagnostic Application.....	45
4. Suggested Therapeutic Applications in Cases without Generally Recognized Hypoprothrombinemia.....	45
VIII. Summary.....	47
References.....	48

I. INTRODUCTION

Following the realization of the clinical significance of vitamin K and the isolation of the vitamin, a number of reviews were published describing the fundamental investigations in this field. (Almquist, 1941; Brinkhous, 1940; Butt and Snell, 1941; Dam, 1942; Dam and Glavind, 1940; Doisy *et al.*, 1941; Koller, 1941; Riegel, 1940.) The present article will deal with the development which has taken place since then.

It must be remarked that when the author wrote a review on Vitamin K several years ago (Dam, 1942), it was possible to present a rather well-rounded picture of all the fundamental facts then available. The information which has been gathered in recent years is much less coherent and comprises several observations (real or postulated) that have been discussed in the literature, without definite conclusion. It would be impossible to mention all the contributions which have appeared, and this review is, therefore, limited to what the author thinks will give a fair representation of the development.

II. VITAMIN K ACTIVE COMPOUNDS

Some new substances have been added to the list of vitamin K active compounds¹ during recent years, some of these are: 1-acetoxy-2-methyl-4-naphthyl sodium phosphate and the corresponding sulfate (Baker and Carlson, 1942; Baker *et al.*, 1942). The compound which is formed by boiling menadione with sodium hydrogen bisulfite (Moore, 1941) has been identified as menadione bisulfite (*J. Am. Med. Assoc.*, 1943). E. Chu (Chu, 1945) states that 4-(3'-methyl-4'-hydroxynaphthylazo)-benzenesulfonamide is almost as potent as menadione,² and 5-methyl-4,7-

¹ Most of the previously known vitamin K active compounds are listed by earlier reviewers, such as Dam (1942) or Rosenberg (1942).

² The committee on nomenclature of the American Medical Association in 1941 (*J. Am. Med. Assoc.*, 1942) adopted the term "menadione" and authorized its use as a nonproprietary name to describe the substance 2-methyl-1,4-naphthoquinone. The U.S.P. used this term for the first time in its XIIth edition. The two naturally

thionaphthenequinone, an isostere of menadione, has been found to have about 3% of the vitamin K activity of menadione (Tarbell *et al.*, 1945). This latter finding is rather peculiar since it might be expected that this compound might antagonize vitamin K.

Mikhlin (Mikhlin, 1942; 1943) has reported that corn stigma contains a substance, termed by him vitamin K₃, which accelerates blood coagulation in normal animals and in patients. It is said to be alcohol-soluble and water insoluble; however, its differentiation from vitamin K₁ is not clear. Other workers have used the term K₃ to designate menadione.

Mentzer and Meunier (Mentzer and Meunier, 1943; 1944) claimed that 3-methylchromone and 2-carboxy-3-methylchromone had a considerable vitamin K activity. Glavind (1948) could not substantiate this claim.

Vitamin K₂ previously isolated from putrified proteins has been found in cultures of a spore-forming soil bacillus, *Bacillus brevis* (Tishler and Sampson (1948)).

III. METHODS FOR THE DETERMINATION OF VITAMIN K AND RELATED SUBSTANCES

1. Blood Clotting Tests

The chick assay has been reexamined by Quick and Stefanini (1948) and a test with rats fed a diet containing sulfadiazine has been proposed by Kornberg *et al.* (1944a, 1944b) (cf. VI, 7).

2. Chemical Tests

Chemical tests have received particular attention. Several reactions, more or less specific for quinones of the vitamin K type, have been proposed as assay methods, but methods for extracting small, i.e., physiological, quantities of vitamin K or its substitutes from biological material do not seem to have been sufficiently worked out.

The following reactions have been proposed:

A. COLORIMETRIC METHODS

Reactions with Dinitrophenylhydrazine (Novelli, 1941; Novelli and Conticello, 1944; Menotti, 1943; Bosecke and Laves, 1943; Vonesch, 1941, 1942a, 1942b, 1942c, 1943; Tastaldi *et al.*, 1945; Giral and Iglesias, 1942; Armstrong, 1943, 1944). The modification by Menotti will be described briefly.

occurring K vitamins: K₁ and K₂ are 3-phytylmenadione and 3-difarnesylmenadione respectively.

To an alcoholic solution of menadione is added a solution of dinitrophenylhydrazine in aqueous hydrochloric acid. By heating to 70°C. for 15 minutes a hydrazone is formed. After cooling to room temperature an alcoholic solution of ammonia is added and the mixture diluted with alcohol to a fixed volume. The reaction results in a blue color which, together with the yellow color of the reagent, makes the solution appear green. The maximum absorption is at 6350 Å. For the details see the publication by Menotti (1943).

The color develops with methylnaphthoquinone but not with vitamin K₁. The method of Menotti is said to be reliable for the determination of 2-methyl-1,4-naphthoquinone in quantities as low as 0.05 mg. The color is very stable.

Craven's Reaction (1931) with Ethyl Cyanoacetate. This has been used by Pinder and Singer (1940) and Kofler (1945) for the determination of methylnaphthoquinone with which it gives a blue color; no color is obtained with vitamin K₁. The method is less sensitive than the reaction with dinitrophenylhydrazine. Fieser (1941) has briefly examined the reaction.

The Reactions with Sodium Diethyl Dithiocarbamate (Irreverre and Sullivan, 1941). With vitamin K₁ a blue color develops while methylnaphthoquinone gives a pink color. The blue color is not stable, it reaches a maximum 7 minutes after the reagents have been mixed, whereafter it fades. The sensitivity is said to be about 5γ/ml.^{2a}

Reactions with Sodium Ethylate (Dam *et al.*, 1939; Karrer, 1939; Fieser, *et al.*, 1939). Vitamin K₁ and vitamin K₂ both give a blue color which later changes to red-brown. Methylnaphthoquinone gives a green color which also changes to red-brown. A variation of this method, using sodium methylate instead of ethylate, has been used by Almquist and Klose (1939). Fernholz *et al.* (1939), used the reaction with sodium methylate and suggested that there might be some vitamin K active substance in plant material which does not give the color reaction. Kofler (1946) states the opposite, viz., that plant material contains another quinone which gives a strong blue color with sodium ethylate and has a very low activity, if any at all, as vitamin K. Bosecke and Laves (1943) have used the method for the determination of methylnaphthoquinones using the Pulfrich *Stufenphotometer*, filters S 53 and S 50, and 2 hours reaction time. The method is less sensitive than the reactions with dinitrophenylhydrazine and diethyl dithiocarbamate.

Carrara *et al.* (1940; 1941) have used an alcoholic solution of sodium hydroxide instead of sodium ethylate. Colucci (1946) has modified the reaction so that it can be used for methylnaphthoquinone or methylnaphthohydroquinone diacetate in oil solution.

^{2a} γ = micrograms.

Other Color Reactions. Methyl-naphthoquinone reacts with 2,6-dichlorophenolindophenol and sodium hydroxide yielding first a brown solution which turns green after shaking. The green color can be taken up in amyl alcohol (Bosecke and Laves, 1943).

Martinson and Meerovich (1945) and Meerovich (1946a; 1946b) report that methyl-naphthoquinone gives a red color on addition of aniline. He used this color for quantitative determination (the reaction has also been described by Fieser, 1941).

Berlin (1940) indicates a procedure whereby methyl-naphthohydroquinone diacetate in methylacetamide gives a red color on addition of sodium hydroxide. He records the time from the addition of sodium hydroxide to the appearance of the red color. The recorded time is inversely proportional to the concentration of the methyl-naphthohydroquinone diacetate. If methyl-naphthoquinone or methyl-naphthohydroquinone is used instead of the diacetate the color appears almost instantaneously. The method can be used for the determination of methyl-naphthoquinone in pharmaceutical preparations. The smallest amount determined seems to be 750 γ /ml.

Greco and Argenziano (1946) propose a reaction with phenyl hydrazine whereby an azo derivative not a phenyl hydrazone is obtained. This derivative, dissolved in sulfuric or phosphoric acid, gives a red-violet color that can be measured in the colorimeter. The method seems to be useful for vitamin K₁ and methyl-naphthoquinone.

Schulek and Rózsa (1941a; 1941b; 1941c; 1941d; 1942) have observed a color reaction between methyl-naphthoquinones and strong acids (concentrated phosphoric acid, 50% sulfuric acid, 20-38% hydrochloric acid). Heating on a water bath gives a red color. The red substance separates on cooling and can be taken up again in various solvents. The method is not very sensitive.

Scudi and Buhs (1941) have introduced a rapid colorimetric method for the determination of menadione. The method consists in the development of a yellow color when cysteine and sodium hydroxide are added to a solution of menadione. The solution may be alcoholic, aqueous or ether-alcoholic, or blood plasma. The method seems also to be applicable to urine and seems to be useful for concentrations as low as 1 or 2 γ /ml. It can also be used for aqueous solutions of 2-methyl-1,4-naphthoquinone-3-sulfonate.

Menotti (1942) has indicated a colorimetric method for the determination of 4-amino-2-methyl-1-naphthol, a vitamin K active pharmaceutical preparation which does not occur in nature. This method is based on the reaction between sodium pentacyanoammineferroate and the before mentioned compound in alcohol solution, whereby a blue color is obtained.

The sensitivity is said to be low, 0.5 γ . Methyl-naphthoquinone gives no color.

Fieser (1941) has examined several color reactions for methyl-naphthoquinone and found that only two of them can be used also for vitamin K₁. These two reactions are those with ethyl cyanoacetate (Craven, 1931) and ethanolamine. Other substances which according to Fieser react with methyl-naphthoquinone are: ethyl acetoacetate, pyruvic acid, aniline (cf. Meerovich 1946a, 1946b) ethanolamine, lysine, thioglycolic acid, and cysteine (cf. Scudi and Buhs, 1942b).

B. REDOX METHODS

Pinder and Singer (1940) have proposed to determine methyl-naphthoquinone by titration with titanous chloride using potassium indigo bisulfonate as indicator.

Trenner and Bacher (1941) have proposed another redox method for vitamin K₁ and methyl-naphthoquinone. This method consists of two stages; viz., the catalytic reduction of the quinone to hydroquinone and reoxidation of an aliquot of the latter with 2,6-dichlorophenolindophenol. They indicate that the highest accuracy (about 1%) is obtained with amounts of K₁ of about 500 to 1000 γ /ml. Using suitable microglass equipment it should be possible to work with as little as 20 γ /ml. The method requires special glass equipment which makes it possible to carry out the catalytic reduction, and the titration with 2,6-dichlorophenolindophenol without exposing the substance to air.

According to Schulek and Rózsa (1941c; 1941d; 1942) methyl-naphthoquinone can be determined by cerimetric titration. The quinone is first reduced to hydroquinone with a solution of stannous chloride in hydrochloric acid, the hydroquinone is extracted with chloroform and titrated with a cerium sulfate solution. *p*-Ethoxychrysoidine serves as indicator. Another redox method has been described by Rosin *et al.* (1941).

C. COLORIMETRIC REDOX METHODS

Scudi and Buhs (1941; 1942a) and Scudi (1941) have proposed a colorimetric redox method based on the principle used by Trenner and Bacher (1941). The method can be used for vitamin K₁ and for methyl-naphthoquinone. The quinone dissolved in butanol is reduced catalytically in the presence of phenosaphranine. To the hydroquinone thereby formed is added an excess of 2,6-dichlorophenolindophenol in butanol under the exclusion of oxygen. The decreasing color intensity is then a measure for the amount of quinone originally present. The method can be used for solutions containing as little as 5 γ K₁/ml. It

requires the same special glass apparatus as the method of Trenner and Bacher.

D. POLAROGRAPHY

Hershberg *et al.* (1940) state that they can determine vitamin K₁ by means of the polarograph in concentrations as low as 50 γ in a solution of 2.5 ml. acetone containing 10 mg. LiCl.

E. FLUOROMETRY

According to Kofler (1945) methylnaphthoquinone can be determined fluorometrically, since it forms a fluorescent compound by condensation with *o*-phenylenediamine. The blue fluorescence of an alcoholic solution of this compound is so intense that it can be detected in solutions down to 0.1 γ in 5 ml. alcohol.

F. SPECTROSCOPIC METHOD

The spectroscopic method has been given renewed attention by Ewing *et al.* (1943).

The following two publications on the determination of vitamin K were not available to the reviewer before the article was finished: Zakharova and Devyatnin (1944) and Schoen (1945).

IV. MODE OF ACTION OF VITAMIN K IN PROTHROMBIN FORMATION

The way in which vitamin K contributes to the formation of prothrombin has been considered largely from the speculative point of view.

Based on the earlier papers of Baumberger (1941), and Bernheim and Bernheim (1940), in which a possible role of thiol groups in fibrin formation was discussed, Lyons (1945) formulated a definite scheme for the mode of action of vitamin K. According to this scheme vitamin K is a functional part of the thrombin molecule and the clotting of fibrinogen occurs in two stages: 1) the liberation of blocked thiol groups in fibrinogen brought about by one component of thrombin, and 2) an oxidation, probably by a naphthoquinone complex in thrombin, converting protein—SH into protein —S—S— protein (fibrin). Lyons claims to have demonstrated the presence of a naphthoquinone derivative in thrombin by means of Novelli's (1941) reaction and Craven's ethyl cyanoacetate test (1931).

Attempts to confirm this latter finding in the reviewer's laboratory have turned out negatively.

Chargaff and Bendich (1943) have shown that a fibrinogen solution may clot on addition of several substances such as chloramine T, potassium 1,4-naphthoquinone-2-sulfonate, sodium 1,2-naphthoquinone-4-sulfonate, ninhydrine, alloxan, and salicylaldehyde while two sulfonic acids

of 2-methyl-1,4-naphthoquinone were inactive. They do not claim, however, that these observations explain the mode of action of vitamin K.

The relation of vitamin K to the two components of prothrombin, whose existence was maintained by A. J. Quick (1943) (cf. the criticism by Seegers *et al.* (1945)), should be investigated as well as its relation to the factor V in blood coagulation described by Owren (1947) and the factor in plasma which accelerates the activation of prothrombin described by Ware *et al.* (1947). Preliminary steps in this direction have been made by Quick (1943), by Dam (1948), and by Dan and S  ndergaard (1948).

The question whether large doses of vitamin K can raise the prothrombin level above normal has been dealt with by Field and Link (1944). They report that the oral administration of large doses of menadione (5 to 20 mg./kg.) to dogs, rabbits, and rats result in hyperprothrombinemia persisting for several days depending on the dose.

Quick (1946) denied that large doses of menadione, synkayvite etc. produce hyperprothrombinemia.

Field *et al.* (1944) also reported that hyperprothrombinemia could be induced by methylxanthine.

An important problem is whether vitamin K and its substitutes are converted into menadione in the body, in which case the latter substance may be looked upon as the true prothrombinogenic factor. Richert (1944) attacked this problem by testing the urine of fowls and rabbits for menadione after the animals had been given certain menadione derivatives, orally or parenterally, in rather large doses. The substances tested were: 4-amino-2-methyl-1-naphthol, 2-methyl-1-tetralone, 2-methyl-1,4-naphthohydroquinone diphosphate. Craven's ethyl cyanoacetate color test was used for the determination of menadione in the urine. A smaller or larger fraction of all the compounds listed was recovered in the urine as menadione.

Meerovich (1946a; 1946b) found that when the bisulfite derivative of 2-methyl-1,4-naphthoquinone, "Vitanol," was injected into the vena jugularis of rabbits some menadione could be found in the urine. Meerovich used the color reaction with cysteine and alkali (Scudi and Buhs, 1942b; Fieser, 1941) for the determination of menadione plus the bisulfite compound, and the reaction with aniline for the determination of menadione.

Whether vitamin K₁ and K₂ are also converted into menadione in the body does not seem to have been definitely settled.

The assertion by some authors (Shemiakin *et al.*, 1943) that phthalic acid should be the intermediary compound which carries the prothrombinogenic effect has been refuted by Dam (1942), Karrer and Koller (1943), and Blumberg and Arnold (1944).

V. EFFECTS OF VITAMIN K AND RELATED SUBSTANCES ON OTHER PROCESSES OTHER THAN PROTHROMBIN FORMATION

1. Possible Influence on Bleeding Tendency in Other Ways than by Prothrombin Formation

An interesting observation on factors which may influence the occurrence of hemorrhages in vitamin K deficient animals has been made by Brown *et al.* (1947). They reared female rats to maturity on an artificial diet deficient in vitamin K. If lard was removed from the diet and the females allowed to bear litters, brain hemorrhages occurred in the offspring. If the diet contained either lard or vitamin K the hemorrhages did not occur. They do not ascribe this observation to vitamin K occurring in the lard but suggest that some substance which acts normally to maintain capillary strength is not synthesized by the body when the diet is low in fat and vitamin K is absent. The coagulation time of the whole blood of females that produced hemorrhagic offspring and of young with visible hemorrhages was said to be normal.

A possible influence of vitamin K on serum antithrombin is mentioned in section VII, 1.

2. Postulated Influence on Blood Pressure

Oppenheimer *et al.* (1944) report that certain quinones, including menadione, have more or less antipressor properties when tested in hypertensive animals. They do not consider this effect specific for vitamin K, however, and state that in the case of menadione rather large doses must be given. Findings similar to these have been published independently by Schwarz and Ziegler (1944).

Moss and Wakerlin (1946) did not find any effect of vitamin K in daily doses of 60 mg. for 6 months in experiments with dogs rendered hypertensive by the Goldblatt technique. A further negative report has been published by Ferregra (1944).

3. Postulated Effect on Proteolytic Enzymes

According to Harkevitch (1942) vitamin K exerts a definite activating action upon trypsin.

4. Postulated Lipotropic Effect

Honorato and García Merino (1942-43) found fatty degeneration of the liver in vitamin K deficient chicks and attributed lipotropic properties to menadione analogous to the properties of choline. A reinvestigation by Field and Dam (1945) failed to confirm this assertion.

5. Influence on Metabolic Processes and Growth of Heterotrophic Organisms

Summerson (1943) examined the effect of menadione on the metabolism of isolated animal tissue. He found that in the presence of this substance in a concentration of approximately 10^{-4} M, rabbit exudate leucocytes respiring in Ringer's bicarbonate-glucose medium show an inhibition of aerobic lactic acid formation of 50% or more; at the same time there was a 10–15% increase in the rate of oxidative metabolism and a slight rise in R.Q. Rabbit bone marrow slices were found to show the same general effect. Rat liver slices also showed increased oxygen uptake while the utilization of lactate diminished. There was no change in R.Q. Under anaerobic conditions the conversion of added glucose to lactic acid by leucocytes was completely inhibited by menadione but the endogenous glycolysis of leucocytes and liver was not affected. The effect of menadione was found to fall off with time, perhaps due to inactivation by combination with cell proteins, presumably their SH groups, since the yellow protein-menadione complex obtained from leucocytes was found to be spectrophotometrically similar to the yellow compound formed by the interaction of menadione and cystein.

Scudi (1942) found that menadione but not vitamin K₁ causes formation of methemoglobin when added to whole blood.

Studies on the inhibitory effect of quinones, including menadione, on lactic acid formation by bacteria and bacteriostatic effect of such quinones have been published by Armstrong and Knutson (1943) and Armstrong *et al.* (1943). These studies are dealt with in this review in the section treating the postulated effect of vitamin K-like substances on dental caries.

According to Atkins and Ward (1945) Gram-negative organisms and a few streptococci are less susceptible to the antibacterial effects of vitamin K analogues than the majority of Gram-positive organisms.

Gonzalez (1945) compared the antibiotic effect of certain quinones on *Penicillium notatum* and found menadione more effective than benzoquinone and hydroquinone. The mechanism of the antibacterial action of quinones and hydroquinones were studied by Geiger (1946). Naphthoquinones as antagonists of the anti-streptococcal action of iodinin (di-*N*-oxide of a dihydroxyphenazine) were studied by McIlwain (1943).

2-Methyl-4-amino-1-naphthol hydrochloride, designated vitamin K₈ by some workers, has been found to possess antibiotic properties towards various fungi and to check fermentation of glucose by yeast (Pratt *et al.*, 1948). The effect is ascribed to altered redox potentials. Practical application of this observation has been suggested.

Ball *et al.* (1947) examined the effect of a number of 2-hydroxy-3-

alkylnaphthoquinones on erythrocytes, liver slices, and yeast cells and found them to be very potent inhibitors of respiratory processes, acting below cytochrome c and above cytochrome b in the main chain of respiratory enzymes. These substances, which include phthiocol, are much less active than vitamin K or than menadione and it cannot be said at present whether these findings have any relation to vitamin K.

Schmidt and Büsing (1942) have published a report according to which a principal source of vitamin K (viz., *Escherichia coli*) grows slowly in the intestine of chicks fed a vitamin K-free diet, and that vitamin K restores the coli flora. This report awaits further confirmation.

It has previously been reported that the growth of *Mycobacterium paratuberculosis* (Johne's bacillus) is greatly stimulated by naphthoquinone derivatives of vitamin K type (Woolley and McCarter, 1940). In an investigation on factors influencing the growth of Johne's bacillus Glavind and Dam (1948) could not find any growth stimulating effect of such naphthoquinones and concluded that the growth stimulating factor which is present in tuberculin, for instance, must be different from vitamin K. The strains of Johne's bacillus used by the two groups of workers were not the same.

6. Influence on Photosynthesis, Respiration, etc. in Photosynthesizing Microorganisms

The role of vitamin K in photosynthesis has received some attention. Dam (1944) found vitamin K in the green alga *Chlorella vulgaris* and also in some photosynthesizing bacteria, although the latter contained less than *Chlorella*. Menadione, added as powder to photosynthesizing *Chlorella*, stopped photosynthesis after having remained in contact with the organism for several hours.

Gaffron (1945) working with the algae *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, and *Scenedesmus sp. D₃*, tested under varied conditions the effects of menadione and phthiocol as well as water-soluble esters of menadione on photosynthesis, respiration, photoreduction, and oxyhydrogen reaction. He found that there is hardly a metabolic process in algae which cannot be influenced by small amounts of the two vitamin K-like substances. Small concentrations of the two naphthoquinones stimulated respiration, while large concentrations inhibited it. The water-soluble esters had less effect.

With very small doses of the two naphthoquinones it was possible to stimulate respiration without inhibiting photosynthesis but otherwise these compounds (not the water-soluble esters) were found to be very powerful inhibitors of photosynthesis. When the naphthoquinones are used in still higher concentrations (e.g., menadione as powder) they

damage the cells progressively, and respiration and photosynthesis are stopped.

Gaffron classifies the two methylnaphthoquinones as poisons in line with hydroxylamine and *o*-phenanthroline and thinks that they may form menadione compounds with heavy metal catalysts. On the other hand their stimulating effect on respiration and capacity to transfer oxygen differ from those of hydroxylamine and *o*-phenanthroline. They may act as oxidoreduction catalysts and may serve in more than one way in the metabolism of the plant.

7. Effect on Mitosis

F. E. Lehmann *et al.* (1942; 1945) have shown that menadione inhibits mitosis in *Tubifex* eggs. Mitchell and Reuss (1947) experimented with tissue cultures of fibroblasts from the choroid and sclerotic tissues of the chick embryo eye as follows:

Group A: cultures were started with synkayvite (tetrasodium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid) in the medium so as to give a concentration of 3×10^{-6} M.

Group B: cultures were started in the same way but after 18 hours an x-ray irradiation of 244 röntgen was given.

Group C: cultures started without synkayvite and irradiated as B after 18 hours.

Counts of cell division were made 24 hours, 36 hours, and 48 hours after the cultures were started. Group B (synkayvite + x-ray) showed a very considerable reduction (about 90%) of cell division, whereas the cell division was much more in the two other groups.

In another series of experiments the concentration of synkayvite was varied without combination with x-ray treatment. It was found that the mitotic counts declined with increasing concentrations of synkayvite.

Experiments with human patients were tried and were found not inconsistent with the tissue culture experiments although at the present stage of investigation no therapeutic value was claimed.

VI. SUBSTANCES CAUSING HYPOPROTHROMBINEMIA AND THEIR RELATION TO VITAMIN K

1. Dicumarol

Substances that cause a lowering of prothrombin, which can be compensated more or less by large doses of vitamin K, have been studied by several authors. One of the most discussed substances is *dicumarol* (3,3'-methylene-bis(4-hydroxycoumarin)). The prothrombin-lowering effect of dicumarol was known (Roderick, 1931) before the substance was isolated (Campbell and Link, 1941). After the isolation and identification of dicumarol some authors, such as J. Lehmann (1942), assumed that

dicumarol acts by a competitive interference with the action of vitamin K; that is, because of a certain structural resemblance to vitamin K, dicumarol should replace vitamin K in some enzyme system involved in the formation of prothrombin. A direct proof for this hypothesis does not seem to have been presented. On the contrary, there are experiments which make this type of explanation seem less likely. Jansen and Jensen (1942) examined a number of compounds representing variations of the dicumarol molecule. In addition to dicumarol they found only 3,3'-ethylidene-bis(4-hydroxycoumarin) active whereas a substance such as 3-methyl-4-hydroxycoumarin, which resembles vitamin K much more closely was inactive. They therefore believe that the effect of dicumarol is not due to a structural resemblance to vitamin K. Glavind and Jansen (1944) determined the amount of dicumarol necessary to overcome the effect of varying doses of simultaneously administered vitamin K (in the form of 2-methyl-1,4-naphthohydroquinone disulfate). They found that the necessary dose of dicumarol increased only from 0.03 to 0.2 (that is, about 7 times) when the amount of vitamin K was increased from 0.00001 to 0.025 (that is, 2500 times). (Both compounds are expressed in terms of milligrams per gram of body weight of the rabbit.) They conclude that this result does not support the replacement theory, since it would be expected that the amount of dicumarol required to replace vitamin K would increase very much (according to the law of mass action) when the dose of the vitamin K compound is increased more than 2000 times. Woolley (1947), aware of Jansen and Glavind's paper, nevertheless seems to favor the replacement theory.

Eriksen *et al.* (1945) found that 3,3'-methylene-bis(3,4-dihydro-4-hydroxycoumarin ("hydrodicumarol")) exerts an action similar to that of dicumarol and suggest that dicumarol is partly converted into hydrodicumarol in the body. They studied the mechanism of the anticoagulant effect of hydrodicumarol and found it to be of a rather complex nature.

Meunier *et al.* (1943) report that methylene-bis(hydroxy-1,4-naphthoquinone) (derived from phthiocol) antagonizes the effect of vitamin K, indicating that the "doubling" of the molecule produces an antagonist.³ This fact might be interpreted in favor of the replacement theory.

³ The words "antagonize" and "antagonist" in this connection are often taken to indicate antagonism due to replacement, but it is not always clear whether an author wishes to have the words so understood. Logically the terms do not necessarily implicate any specific mechanism by which the antagonistic effect is brought about. In cases where the effect of the antagonist is easily overcome by a suitable increase of the dose of vitamin K, and vice versa it is likely that the replacement theory holds, provided that beginning liver damage, or blocking of supply of vitamin K by intestinal bacteria, or interference with absorption from the intestine do not occur.

Meunier and Mentzer (1942) found that the effect of dicumarol is antagonized by 2-methyl-4-hydroxycoumarin, the substance which Jansen and Jensen found did not antagonize vitamin K.

It is worthwhile to remember that dicumarol exerts a toxic effect which manifests itself in other ways than by hypoprothrombinemia; it causes loss of weight and the animals often die without bleeding. Emmel and Dam (1944) did not find any indication of fatty degeneration in the liver of dicumarol-poisoned chicks, but the possibility should be considered that the general intoxication might interfere with prothrombin formation just as it is seen in the initial stages of chloroform poisoning where it is also possible to partially compensate the hypoprothrombinemia by ingestion of vitamin K.

Jansen (1944) believes in the intoxication theory, and reports liver damage in dicumarol poisoned rabbits in accordance with the observations of Wakim *et al.* (1943). The intoxication theory is further supported by the observations on ducklings made by Rigdon and Varnadoe (1947) according to which there seems to be a relationship between the toxicity of dicumarol and the amount of vitamin A in the diet.

Quick and Stefanini (1948) report that vitamin K₁ and menadione are about equally effective in counteracting the hypoprothrombinemic effect of dicumarol.

2. Isosteres of Vitamin K with Antagonistic Action

A rather interesting example of antagonism, viz., that of α -tocopherolquinone, vitamin K, and vitamin E, was studied by Woolley (1945). He found that α -tocopherolquinone opposes the effect of vitamin E and that this antagonistic effect can be abolished by vitamin K but not by vitamin E. Another antagonist to vitamin K is, according to Woolley, 2,3-dichloro-1,4-naphthoquinone.

Smith *et al.* (1946; 1947) studied the antagonistic effect of derivatives of phthiocol with substituents in the methyl group. The hemorrhagic effect of their compounds was noted during the examination of the toxicity of various antimalarial drugs. They found hemorrhagic and toxic effects of such compounds as 2-(3-cyclohexylpropyl)-3-hydroxy-1,4-naphthoquinone (called S 5090), 2-(2-methyloctyl)-3-hydroxy-1,4-naphthoquinone and 2-(3-(decahydro-2-naphthyl)propyl)-3-hydroxy-1,4-naphthoquinone. The effects were antagonized partially by menadione and completely by vitamin K₁. The compounds produced only minimal changes in the liver, caused no destruction of prothrombin *in vitro* and possessed only slight bacteriostatic activity against *Escherichia coli*, the principal source of vitamin K in the intestinal tract. They conclude that these compounds exert their antagonistic effect by competition with

vitamin K. It is noteworthy that they found vitamin K₁ to be more effective in compensating the hemorrhagic effect than menadione.

3. Salicylates

The production of hypoprothrombinemia by the ingestion of salicylates by rats maintained on a ration low in vitamin K, was reported by Link *et al.* (1943). They found that vitamin K in the form of menadione protects the rats against the hypoprothrombinemia induced by salicylic acid and they considered the possibility that degradation of dicumarol to salicylate in the body might explain the hemorrhagic effect of dicumarol. A similar effect of salicylate (and acetylsalicylate) in human patients was noticed by Meyer and Howard (1943), though the increases of prothrombin time and coagulation time were rather small, and by Rapoport *et al.* (1943) and Shapiro *et al.* (1943). According to the latter authors acetylsalicylic acid appears to be a more potent agent than sodium salicylate. Lester (1944) reported that no salicylate appeared in the urine after ingestion of dicumarol by rats. Since the effect of salicylate is much less than the effect of dicumarol it seems unlikely that dicumarol acts via salicylic acid. Jacques and Lepp (1947) report that sodium salicylate changes the prothrombin only when given orally. They suggest that salicylate may be converted to dicumarol or a substance with similar prothrombinopenic properties by bacterial action in the intestinal tract. The use of vitamin K in salicylate therapy is still being considered. Quick (1945) doubted that salicylates produce dangerously lowered prothrombin and suggested that salicylates may cause hemorrhages in other ways, perhaps through thrombocytopenia.

4. Dihydroxystearic Acid

Lockhart *et al.* (1942) found that the feeding of 25% synthetic fat containing dihydroxystearic acid (1 molecule of this acid +2 molecules of other fatty acids in the triglyceride) in vitamin K-deficient rat diets resulted in rapid development of vitamin K deficiency symptoms. Small amounts of menadione prevented the symptoms. This interesting observation was further investigated by Nightingale *et al.* (1947). They found that dihydroxystearic acid blocks the synthesis of vitamin K by intestinal bacteria without killing the bacteria. Thus their observations deal with a case where a substance definitely aids the production of K deficiency without simply acting as a "competitive antagonist" for the vitamin.

5. Vitamin A in Massive Doses

The production of hemorrhages by feeding excessive amounts of vitamin A in the form of either crystalline vitamin A or halibut liver oil

was noted by Rodahl and Moore (1943), Moore and Wang (1945) and by Light *et al.* (1944). The latter authors, as well as Walker *et al.* (1947), found that the condition is associated with hypoprothrombinemia and may be prevented by the simultaneous addition of vitamin K in relatively small amounts.

Quick and Stefanini (1948) confirmed this observation in rats but could not find a similar effect when chicks were used as experimental animals. They point out the possibility that the effect is due to interference with bacterial synthesis of vitamin K.

6. Mineral Oil

Production of vitamin K deficiency in rats by ingestion of large amounts of mineral oil, whereby the intestinal absorption of the vitamin is reduced, was reported in 1940 by Elliott *et al.* and later by Javert and Macri (1941). The paper of the first mentioned authors contained the statement that in addition to vitamin K, activated ergosterol definitely improved the deficiency. This statement does not seem to have attracted sufficient attention.

7. Sulfa Drugs

Kornberg *et al.* (1944a; 1944b) have recommended the use of sulfadiazine in producing vitamin K deficiency in rats for the purpose of biological determination of vitamin K. The sulfadiazine is supposed to act by suppressing the intestinal flora. Granados and Dam (1945) also reared rats on similar diets. While the rats developed vitamin K deficiency they showed severe signs of sulfa-drug poisoning (loss of weight, weakness, hematuria, urinary calculi, and early death) and were scarcely of any value as test animals.

Seeler *et al.* (1944) and Mushett and Seeler (1947) studied the hypoprothrombinemia resulting from the administration of sulfaquinoxaline (2-sulfanil-amido quinoxaline). They found that the hypoprothrombinemic action cannot be attributed solely to either the sulfamido or the quinoxaline portion of the molecule, but is due to the combination of both. They report that vitamin K₁ is many times as potent as menadione in compensating the effect of sulfa quinoxaline.

8. Other Compounds

Procaine used for spinal anesthesia is said to minimize the clotting power of the blood while vitamin K counteracts this effect (Levy and Conroy, 1947).

VII. APPLICATION OF VITAMIN K IN HUMAN MEDICINE

1. *The Well Established Therapeutic Applications of Vitamin K in Cases with Generally Recognized Hypoprothrombinemia*

The well established therapeutical applications of vitamin K against bleeding tendencies in surgical patients with obstructive jaundice, against bleeding tendencies in the newborn, and in celiac disease etc., has been so well described before that it does not seem necessary to go further into details in this review. The following supplementary information may suffice.

Marx and Dyckerhoff (1943) reported that in obstructive jaundice the coagulation anomaly is caused, not only by lack of prothrombin, but also by increased antithrombin. The increase in antithrombin is due to the increase in the affinity of one or more blood proteins for thrombin. Vitamin K is said to influence advantageously not only the prothrombin but also the abnormal condition of the blood proteins.

The exact daily requirement of human adults for vitamin K has not yet been determined, but figures for the newborn are available. Sells *et al.* (1941) reported that the requirement during the first few days after birth is extremely low (1-2 γ of 2-methyl-1-amino-4-naphthol hydrochloride). This claim has largely been substantiated by other investigators. Thus, Larsen (1943) found that 1 γ of 2-methyl-1,4-naphthohydroquinone disuccinate is sufficient for the newborn when given intravenously every day, and that 5 γ of this substance or of pure vitamin K₁ is sufficient when given orally daily.

Sarah H. Hardwicke (1944) working with the tetrasodium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid, "Synkayvite," found the minimal effective daily dose to lie somewhere between 5 and 0.5 γ . If it is intended to produce and maintain normal prothrombin through the first 7-10 days after birth by one single dose, the amount given should be higher.

The cause of the hypoprothrombinemia of the newborn was studied from different points of view (Dam *et al.*, 1942; Glavind *et al.*, 1942; Plum and Uldall, 1942; and Venndt and Plum, 1942) including the possible role of bile acid production, fat digestion, vitamin K content of feces, and vitamin K content of human and cow's milk.

Idiopathic hypoprothrombinemia refractory to vitamin K has been described by Plum (1943a) and by Murphy and Clark (1944). Thorough investigations on the technique for prothrombin determination were made by Plum and Larsen (1942).

Statistics on the value of vitamin K treatment of mothers prior to delivery on the incidence of bleeding and the death rate among the newborn have been published by Beck *et al.* (1941), by Hellman and Shettles (1942), and by Parks and Sweet (1942). Statistics on the treatment of the infant were published by J. Lehmann (1944) and others.

Further investigation on the successful use of vitamin K in gastrointestinal diseases of children has been published by Plum (1943b). Bercovitz and Paye (1944) reinvestigated the occurrence of vitamin K deficiency in case of ulcerative colitis (Clark *et al.*, 1939). They confirmed that vitamin K deficiency may be found in cases of this disease.

The assertion that vitamin K deficiency occurs in patients with pulmonary tuberculosis (Levy, 1942; Sheely, 1941) was reinvestigated by Plum and Poulsen (1942) but was not confirmed, a fact which is in accordance with preliminary investigations by Gyntelberg and Dam (1941).

Marie Stoppelman (1942) reported that vitamin K increases the agglutinating power of serum and argued that blood transfusions should not be given until 48 hours after administration of vitamin K. Subsequent investigators failed to confirm this claim (Gammelgaard *et al.*, 1943; Tronsberg, 1944).

Kinsey (1944) recommends the parenteral ingestion of menadione by blood donors, up to 18 mg. 24 hours before, or 20–30 mg. 48 hours before donation to patients with intrahepatic jaundice although it is stated that the prothrombin content in the blood of the donors remained essentially the same before and after they received vitamin K.

2. Hypoprothrombinemia of Pancreatic Origin

The influence of the pancreas on prothrombin was first studied by Sproul and Sanders (1941) in experiments with cats. The pancreatic duct was cut or pancreatectomy was performed. Hypoprothrombinemia developed in the course of some time. Begtrup and Tage-Hansen (1947) studied plasma prothrombin in dogs before and after pancreatectomy. They found that this form of hypoprothrombinemia was refractory to vitamin K therapy, that it developed about 1 week after the operation was performed and disappeared spontaneously about 3 weeks after the operation. Begtrup (1947, 1948) found a case of pancreatic disease associated with hypoprothrombinemia which could not be alleviated by vitamin K therapy. The patient suffered from a pancreatic tumor, was not jaundiced, and did not show signs of liver damage in the usual tests. The prothrombin was about 40% of the normal value.

3. Diagnostic Application

Hypoprothrombinemia may occur in patients with jaundice due to obstruction as well as in those with jaundice due to hepatitis. In obstructive jaundice the prothrombin will rise to normal values after ingestion of vitamin K in suitable form and quantity whereas in hepatitis the response is only slight. Lord and deWitt Andrus (1941) based a test for liver function on this fact and used it for discrimination between the two types of jaundice. Many other investigators have studied the value of this diagnostic procedure (Koller, 1941; Kark and Souter, 1941; Begtrup and From Hansen, 1942; From Hansen and Begtrup, 1943; Stein, 1944). Kark and Souter presented a detailed discussion of the possible variations of the response in the test and their diagnostic significance. Begtrup and From Hansen stressed the fact that it is unnecessary to give, orally or parenterally, more than 1 mg. of vitamin K preparation such as menadione bisulfite or compounds of similar activity. If the increase in prothrombin 24 hours after the ingestion is 20% of the initial value or less, it is safe to conclude that damage of liver parenchyma is present. Uncomplicated cases of obstruction will give a greater response (usually > 50%) in the same length of time.

4. Suggested Therapeutic Applications in Cases without Generally Recognized Hypoprothrombinemia

The most extensive use of vitamin K therapy has been proposed by A. Palladin (1945). He recommends the use of menadione (10–15 mg. in 0.1% alcoholic solution diluted with an equal amount of water before use) in cases of hemorrhages which are not (or not unanimously) considered due to low prothrombin, such as hemorrhages from granulations, pulmonary hemorrhages from penetrating wounds of the thorax, or from pulmonary tuberculosis; prophylactically against bleeding in connection with tonsillectomy; sequestrectomy in osteomyelitis of maxilla following gunshot wounds; further against bleeding from fresh open wounds, bed sores, frostbites, burns, ulcerous stomatitis. According to Palladin such treatment accelerated healing, shortened convalescence and enabled soldiers to return to the front more speedily; it also influenced capillary hemorrhages present in scurvy. Palladin considers it dangerous to limit the use of vitamin K to cases with low prothrombin and suggests that vitamin K counteracts bleeding in other ways than by raising prothrombin, possibly by raising fibrinogen (cf. Field and Dam, 1946). The reviewer has not been able to find any convincing proof for these sweeping statements and they are not generally accepted as yet. Goldberg and

Milgram (1945) argue the use of vitamin K in cases of hemorrhages after extraction.

Kallós (1946) reports increased prothrombin time in patients with chronic urticaria and angioneurotic edema in whom allergic etiology could not be demonstrated, and mentions that vitamin K therapy seems to have a beneficial effect. These statements still await further investigation.

Vitamin K therapy in menorrhagia has been discussed by Gubner (1944), its use in the treatment of chilblains by Wheatley (1947).

The possible use of the antibiotic compound 2-methyl-4-amino-1-naphthol hydrochloride in clinical treatment of dermato-mycoses has been suggested (Pratt *et al.*, 1948).

A postulated relation of vitamin K and vitamin K like substances to dental problems has been extensively discussed in the literature. Although the reviewer is of the opinion that the results extend beyond the field of vitamin research, it might be of interest to mention some of the contributions which have appeared.

In 1942 Fosdick *et al.* (1942) reported that 1 mg. of menadione in 100 cc. of a saliva-glucose mixture prevented acid formation in a 4-hour incubation period, whereas the same mixture in absence of vitamin K produced up to 2 milliequivalents of acid under the same conditions. In clinical observations the authors found that menadione, in the same concentrations as in the *in vitro* experiments, also inhibited acid formation. The authors suggested that such an inhibitory action of vitamin K was not due to any antiseptic property, and proposed the use of vitamin K in the prevention of dental caries. Armstrong and Knutson (1943) and Armstrong *et al.* (1943), comparing the inhibitory action of 13 quinones, with high, low, and no vitamin K activity, in conditions similar to those in Fosdick's experiments, demonstrated that the inhibitory effect of the various quinones on acid production was due to the bacteriostatic and bactericidal action of the quinone structure, independent of any vitamin K activity. Further, they showed that several of the quinones effective in the prevention of acid formation in incubated saliva-glucose mixtures also inhibited the growth of certain cocci.

Fancher *et al.* (1944) tested the effect of certain substances, including most of the known vitamins, on the rate of acid formation from fermentable sugars in saliva. Only cholesterol, "Cerophyl," and thiamine stimulated acid formation. Both menadione and natural vitamin K₁ inhibited acid formation. There was no indication that this action was related to the vitamin K activity of the compounds. Calandra *et al.* (1944) studied the action of certain derivatives of the quinones on the rate of acid formation in saliva, and all those tested inhibited acid forma-

tion in varying degrees. Menadione proved to have an inhibitory power equal to any of the other quinones tested, and superior to most of them. The mechanism whereby the quinones inhibit acid production and act as antibiotic agents (Geiger, 1946; Hoffman-Ostenhof, 1947) is very complex.

Granados and Dam (1945) studied the influence of vitamin K on the rat incisor, using basal diets containing 1% sulfadiazine for preventing vitamin K production by the intestinal flora. No gross changes in the incisors could be attributed to vitamin K deficiency. A striking growth decrease and a marked decline of eruption and attrition rates were produced by the sulfadiazine content of the rations.

Hodge (1944) and Hatton *et al.* (1945) reported that in albino rats menadione did not decrease the number of carious lesions. In humans, Burrill *et al.* (1945) studied the effect of menadione incorporated into chewing gum, on the occurrence of new carious lesions, and the progress of old ones. In the subjects who chewed the vitamin K containing gum there was a reduction of 60 to 90% in the occurrence of new lesions, but no apparent inhibition in the progress of caries already present. However, the Professional Service Schools (1946) found no differences in the number of new carious lesions exhibited by subjects who had chewed menadione-containing gum as compared with the controls that chewed menadione-free gum.

Thus, though certain quinones, regardless of their vitamin K activity, inhibit the acid formation by, and growth of, certain cocci, menadione does not decrease the incidence of caries in the albino rat, and in humans the results are contradictory. The basis for these contradictions could, at least partially, lie on a misconception of the caries process itself. The optimistic concept on the beneficial role of vitamin K, through its acid-inhibiting power, in dental caries, has been based, so far, on the assumption that the initial lesion is fundamentally a process due to acid action (Fosdick, 1943). The experimental and clinical studies carried out up to the present, do not support this view. The work of certain investigators, reviewed recently by Pincus (1944), Nuckolls and Frisbie (1946), Leicester (1946), and Gottlieb (1947) on the importance of the organic component of the enamel in the initiation of caries, deserves consideration. Any further study of the problem in animal experiments should be carried out with animals that are more suitable for the dietary development of dental caries than the albino rat.

VIII. SUMMARY

The review deals mainly with developments since 1941.

Some new vitamin K compounds have been reported.

A series of color reactions more or less specific for vitamin K active

substances have been proposed but here has been little progress towards making these reactions applicable to biological material.

An elaborate theory for the participation of vitamin K in prothrombin formation has been set forth without convincing experimental proof. The relation of vitamin K to the more advanced concept of blood coagulation has been discussed by some authors.

It has been suggested that vitamin K might act on other biological processes than prothrombin formation. Some of the more promising of the studies within this field seem to be those dealing with the influence of vitamin K-like substances on metabolic processes in tissue or individual cells.

The formation of vitamin K by bacteria has received renewed attention through the demonstration of the blockage of this process by dihydroxystearic acid and, possibly, by massive doses of vitamin A.

The development of hypoprothrombinemia by other means than deprivation of vitamin K as well as the influence of vitamin K and its derivatives on such hypoprothrombinemias has been studied by many authors. Dicumarol and isosteres of vitamin K with antagonistic action have been dealt with extensively. According to several authors the mode of action of dicumarol does not seem to be a simple "replacement antagonism."

The well established therapeutic application of vitamin K in cases with generally recognized hypoprothrombinemia has been studied in detail by many investigators. The same applies to the diagnostic application in liver function tests. In addition thereto a considerable number of medical applications have been suggested in cases without hypoprothrombinemia. The reviewer emphasizes the lack of sufficient evidence for the relevance of vitamin K therapy in many of these cases.

REFERENCES

- Almquist, H. J. 1941. *Physiol. Rev.* **21**, 194-216.
Almquist, H. J., and Klose, A. A. 1939. *J. Am. Chem. Soc.* **61**, 1610.
Armstrong, M. R. 1943. *Anales quim. y farm. Santiago, Chile* 48-52.
Armstrong, M. R. 1944. *Chem. Abstracts* **38**, 2789.
Armstrong, W. D., and Knutson, J. W. 1943. *Proc. Soc. Exptl. Biol. Med.* **52**, 307-310.
Armstrong, W. D., Spink, W. W., and Kahnke, J. 1943. *Proc. Soc. Exptl. Biol. Med.* **53**, 230-234.
Atkins, P., and Ward, J. L. 1945. *Brit. J. Exptl. Path.* **26**, 120-124.
Baker, B. R., and Carlson, G. H. 1942. *J. Am. Chem. Soc.* **64**, 2657-2664.
Baker, B. R., Davies, T. H., McElroy, L., and Carlson, G. H. 1942. *J. Am. Chem. Soc.* **64**, 1096-1101.
Ball, E. G., Anfinsen, Chr. B., and Cooper, O. 1947. *J. Biol. Chem.* **168**, 257-270.
Baumberger, J. P. 1941. *Am. J. Physiol.* **133**, 206.

- Beck, A. C., Taylor, E. S., and Colbarn, R. F. 1941. *Am. J. Obstet. Gynecol.* **41**, 765.
- Begtrup, H. 1947. *Acta Med. Scand.* **129**, 33-36.
- Begtrup, H. 1948. *Acta Med. Scand.* **131**, 95.
- Begtrup, H., and Hansen, P. From. 1942. *Nord. Med.* **14**, 1851-1855.
- Begtrup, H., and Tage-Hansen, E. 1947. *Acta Physiol. Scand.* **14**, 189-194.
- Bercovitz, Z., and Paye, R. S. 1944. *Ann. Internal Med.* **20**, 239-253.
- Berlin, H. 1940. *Svensk Kem. Tid.* **52**, 233-238.
- Bernheim, F., and Bernheim M. 1940. *J. Biol. Chem.* **134**, 457-458.
- Blumberg, H., and Arnold, A. 1944. *Proc. Soc. Exptl. Biol. Med.* **57**, 255-256.
- Bosecke, W., and Laves, W. 1943. *Biochem. Z.* **314**, 285-290.
- Brinkhous, K. M. 1940. *Medicine* **19**, 329-416.
- Brown, E. E., Fudge, J. F., and Richardson, L. R. 1947. *J. Nutrition* **34**, 141-152.
- Burrill, D. Y., Calandra, J. C., Tilden, E. B., and Fosdick, L. S. 1945. *J. Dental Research* **24**, 273-282.
- Butt, H. R., and Snell, A. M. 1941. Vitamin K. Saunders, Philadelphia and London.
- Calandra, J. C., Fancher, O. E., and Fosdick, L. S. 1944. *J. Dental Research* **23**, 31-37.
- Campbell, H. A., and Link, K. P. 1941. *J. Biol. Chem.* **138**, 21-33.
- Carrara, G., Braidotti, L., and Guidarini, C. 1940. *Chimica e industria Milan* **22**, 317-321.
- Carrara, G., Braidotti, L., Guidarini, C. 1941. *Chem. Abstracts* **35**, 1836.
- Chargaff, E., and Bendich, A. 1943. *J. Biol. Chem.* **149**, 93-110.
- Chu, Edith Ju Hwa. 1945. *J. Am. Chem. Soc.* **67**, 811-812.
- Clark, C. L., Dixon, C. E., Butt, H. R., and Snell, A. M. 1939. *Proc. Staff Meetings Mayo Clinic* **14**, 407.
- Colucci, D. B. 1946. *Chem. Abstracts* **40**, 6527.
- Craven, A. 1931. *J. Chem. Soc.* 1605-1606.
- Dam, H. 1942. *Advances in Enzymol.* **2**, 285-324.
- Dam, H. 1943. *Nature* **152**, 355.
- Dam, H. 1944. *Am. J. Botany* **31**, 492-493.
- Dam, H. 1948. *Nature* **161**, 1010.
- Dam, H., Geiger, A., Glavind, J., Karrer, P., Karrer, W., Rothschild, E., and Salomon, H. 1939. *Helv. Chim. Acta* **22**, 310-313.
- Dam, H., and Glavind, J. 1940. *Enzymologia* **9**, 215-227.
- Dam, H., Glavind, J., Larsen, E. Hj., and Plum, P. 1942. *Acta Med. Scand.* **112**, 210-216.
- Dam, H., and Søndergaard, E. 1948. *Biochim. et Biophys. Acta.* **2**, 409-413.
- Doisy, E. A., Binkley, S. B., and Thayer, S. A. 1941. *Chem. Revs.* **28**, 477-517.
- Elliott, Margaret C., Isaacs, B. L., and Ivy, A. C. 1940. *Proc. Soc. Exptl. Biol. Med.* **43**, 240-245.
- Emmel, J. M., and Dam, H. 1944. *Proc. Soc. Exptl. Biol. Med.* **56**, 11-14.
- Eriksen, F., Jacobsen, E., and Plum, C. M. 1945. *Acta Pharmacol. Toxicol.* **1**, 379-393.
- Ewing, D. T., Tomkins, F. S., and Kamm, O. 1943. *J. Biol. Chem.* **147**, 233-241.
- Fancher, O. E., Calandra, J. C., and Fosdick, L. S. 1944. *J. Dental Research* **23**, 23-29.
- Fernholz, E., Ansbacher, S., and Moore, M. 1939. *J. Am. Chem. Soc.* **61**, 1613-1614.
- Ferregra, A. B. 1944. *Rev. asoc. med. argentina* **58**, 163-165.
- Field, J. B., and Dam, H. 1945. *Proc. Soc. Exptl. Biol. Med.* **60**, 146-148.

- Field, J. B., and Dam, H. 1946. *J. Nutrition* **31**, 509-523.
- Field, J. B., Larsen, E. G., Spero, L., and Link, K. P. 1944. *J. Biol. Chem.* **156**, 725-737.
- Field, J. B., and Link, K. P. 1944. *J. Biol. Chem.* **156**, 739-741.
- Fieser, L. F. 1941. *Ann. Internal Med.* **15**, 648-658.
- Fieser, L. F., Campbell, W. P., and Fry, E. M. 1939. *J. Am. Chem. Soc.* **61**, 2206-2218.
- Fosdick, L. S. 1943. *J. Can. Dental Assoc.* **9**, 359.
- Fosdick, L. S., Fancher, O. E., and Calandra, J. C. 1942. *Science* **96**, 45.
- Gaffron, H. 1945. *J. Gen. Physiol.* **28**, 259.
- Gammelgaard, A., Larsen, E. Hj., and Marcussen, P. V. 1943. *Acta Med. Scand.* **116**, 8-10.
- Geiger, W. B. 1946. *Arch. Biochem.* **11**, 23-31.
- Giral, F., and Iglesias, S. 1942. *Ciencia Mex.* **3**, 157-159.
- Glavind, J. 1948. Personal communication.
- Glavind, J., and Dam, H. 1948. *Physiologia Plantarum* **1**, 1-4.
- Glavind, J., and Jansen, K. F. 1944. *Acta Physiol. Scand.* **8**, 173-182.
- Glavind, J., Larsen, E. Hj., and Plum, P. 1942. *Acta Med. Scand.* **112**, 198-209.
- Goldberg, M. S., and Milgram, E. Y. 1945. *Am. Rev. Soviet. Med.* **2**, 272.
- Gonzalez, Felipe. 1945. *Science* **101**, 494-495.
- Gottlieb, B. 1947. Dental Caries. Lea & Febiger, Philadelphia.
- Granados, H., and Dam, H. 1945. *J. Dental Research* **24**, 137-139.
- Greco, D., and Argenziano, R. 1946. *Chem. Abstracts* **40**, 7516.
- Gubner, R. 1944. *Southern Med. J.* **37**, 556-558.
- Gyntelberg, J., and Dam, H. 1941. *Ugeskrift Laeger* **103**, 263-264.
- Hansen, P. From, and Begtrup, H. 1943. *Acta Med. Scand.* **113**, 1-10.
- Hardwicke, Sarah Hooker. 1944. *J. Pediat.* **24**, 259-269.
- Harkevitch, N. 1942. *Sperimentale* **96**, 611-614, quoted through *Chem. Centr.* (1943) ii, p. 1892.
- Hatton, E. H., Dodds, A., Hodge, H. C., and Fosdick, L. S. 1945. *J. Dental Research* **24**, 283-295.
- Hellman, L. M., and Shettles, L. B. 1942. *Southern Med. J.* **35**, 289-293.
- Hershberg, E. B., Wolfe, J. K., and Fieser, L. F. 1940. *J. Am. Chem. Soc.* **62**, 3516-3518.
- Hodge, H. C. 1944. *J. Dental Research* **23**, 207.
- Hoffman-Ostenhof, O. 1947. *Science* **105**, 549.
- Honorato, C. R., and García Merino, V. 1942-43. *Rev. med. y aliment. Santiago, Chile* **5**, 139-141.
- Irreverre, F., and Sullivan, M. X. 1941. *Science* **94**, 497-498.
- Jacques, L. B., and Lepp, Erica. 1947. *Proc. Soc. Exptl. Biol. Med.* **66**, 178-181.
- J. Am. Med. Assoc.* **120**, 226 (1942).
- J. Am. Med. Assoc.* **121**, 839 (1943).
- Jansen, K. F. 1944. Dikumarin. Thesis, Copenhagen, Ejnar Munksgaard, Copenhagen.
- Jansen, K. F., and Jensen, K. A. 1942. *Z. physiol. Chem.* **277**, 66-73.
- Javert, Carl T., and Macri, C. 1941. *Am. J. Obstet. Gynecol.* **42**, 409-414.
- Kallós, P. 1946. *Gastroenterologia* **71**, 1-4.
- Kark, R., and Souter, A. W. 1941. *Lancet* 693.
- Karrer, P. 1939. *Helv. Chim. Acta* **22**, 1146-1149.
- Karrer, P., and Koller, F. 1943. *Helv. Chim. Acta* **26**, 2114-2115.

- Kinsey, Roy E. 1944. *Arch. Internal Med.* **73**, 131-137.
- Kofler, M. 1945. *Helv. Chim. Acta* **28**, 702-713.
- Kofler, M. 1946. *Festschrift E. Barell*, Basel.
- Koller, F. 1941. *Das Vitamin K und seine klinische Bedeutung*. G. Thieme, Leipzig.
- Kornberg, A., Daft, F. S., and Sebrell, W. H. 1944a. *Public Health Reps.* **59**, 832-844.
- Kornberg, A., Daft, F. S., and Sebrell, W. H. 1944b. *J. Biol. Chem.* **155**, 193-200.
- Larsen, E. Hj. 1943. *Nord. Med.* **17**, 257-258.
- Lehmann, F. E. 1942. *Verhandl. Ver. schweiz. Physiol.* 24-25.
- Lehmann, F. E., Lüscher, M., and Huber, W. 1945. *Rev. suisse zool.* **52**, 342, 349, 354.
- Lehmann, J. 1942. *Svenska Läkartidn.* **39**, 73-79.
- Lehmann, J. 1944. *Lancet* **246**, 483-494.
- Leicester, H. M. 1946. *Ann. Rev. Biochem.* **15**, 361-374.
- Lester, D. 1944. *J. Biol. Chem.* **154**, 305-306.
- Levy, S. 1942. *Am. Rev. Tuberc.* **45**, 377.
- Levy, S., and Conroy, L. 1947. *Med. Record* **160**, 96-99. Quoted from *Excerpta Medica* **1**, 1068, 1948.
- Light, R. F., Alscher, R. P., and Frey, C. N. 1944. *Science* **100**, 225-227.
- Link, K. P., Overman, R. S., Sullivan, W. R., Huebner, Chr. F., and Scheel, L. D. 1943. *J. Biol. Chem.* **147**, 463-474.
- Lockhart, E. E., Sherman, H., and Harris, R. S. 1942. *Science* **96**, 542-543.
- Lord, J. W., Jr., and Andrus, W. D. 1941. *Arch. Internal Med.* **68**, 199-210.
- Lyons, R. N. 1945. *Australian J. Exp. Biol. Med. Sci.* **23**, 131-140.
- Martinson, E. E., and Meerovich, G. I. 1945. *Biokhimiya* **10**, 258.
- Marx, R., and Dyckerhoff, H. 1943. *Klin. Wochschr.* **22**, 570.
- McIlwain, H. 1943. *Biochem. J.* **37**, 265-271.
- Meerovich, G. I. 1946a. *Biokhimiya* **11**, 45-52.
- Meerovich, G. I. 1946b. *Chem. Abstracts* **40**, 5791.
- Menotti, A. R. 1942. *Ind. Eng. Chem., Anal. Ed.* **14**, 601-602.
- Menotti, A. R. 1943. *Ind. Eng. Chem., Anal. Ed.* **15**, 418-420.
- Mentzer, C., and Meunier, P. 1943. *Bull. soc. chim. France*, Ser. 5 **10**, 126.
- Mentzer, C., and Meunier, P. 1944. *Bull. soc. chim. France*, Ser. 5 **10**, 405.
- Meunier, P., and Mentzer, C. 1942. *Bull. soc. chim. biol.* **24**, 371-375.
- Meunier, P., Mentzer, C., Hoi, Buu, and Cagniant, P. 1943. *Bull. soc. chim. biol.* **25**, 384-390.
- Meyer, O. O., and Howard, B. 1943. *Proc. Soc. Exptl. Biol. Med.* **53**, 234-237.
- Mikhlin, D. M. 1942. *Compt. rend. acad. sci. URSS* **37**, 191-192.
- Mikhlin, D. M. 1943. *Biokhimiya* **8**, 158-167.
- Mitchell, J. S., and Reuss, J. S. 1947. *Nature* **160**, 98-99.
- Moore, B. 1941. *J. Am. Chem. Soc.* **63**, 2049.
- Moore, T., and Wang, Y. L. 1945. *Biochem. J.* **39**, 222-228.
- Moss, W. G., and Wakerlin, G. E. 1946. *J. Pharmacol. Exptl. Therap.* **86**, 355.
- Murphy, T. D., and Clark, J. K. 1944. *Am. J. Med. Sci.* **207**, 77-83.
- Mushett, C. W., and Seeler, A. O. 1947. *J. Pharmacol. Exptl. Therap.* **91**, 84-91.
- Nightingale, G., Lockhart, E. E., and Harris, R. S. 1947. *Arch. Biochem.* **12**, 381-387.
- Novelli, A. 1941. *Science* **93**, 358.
- Novelli, A., and Conticello, J. S. 1944. *J. Am. Chem. Soc.* **66**, 842.

- Nuckolls, J., and Frisbie, H. E. 1946. *J. Am. Coll. Dentists* **13**, 84-100.
- Oppenheimer, B. S., Soloway, S., and Lowenstein, B. E. 1944. *J. Mt. Sinai Hosp.* **11**, 23-27.
- Owren, P. A. 1947. The Investigation of a New Clotting Factor. J. Chr. Gunder-
sen, Oslo.
- Palladin, A. 1945. *Am. Rev. Soviet Med.* **2**, 267-269.
- Parks, J., and Sweet, L. K. 1942. *Am. J. Obstet. Gynecol.* **44**, 432-442.
- Pincus, P. 1944. *Brit. Dental J.* **76**, 231-240.
- Pinder, J. L., and Singer, J. H. 1940. *Analyst* **65**, 7-12.
- Plum, P. 1943a. *Ugeskrift Laeger* **105**, 51-59.
- Plum, P. 1943b. *Acta Med. Scand.* **113**, 262-265.
- Plum, P., and Larsen, E. Hj. 1942. *Nord. Med.* **16**, 3407-3427.
- Plum, P., and Poulsen, J. E. 1942. *Acta Med. Scand.* **112**, 426-434.
- Plum, P., and Uldall, C. 1942. *Acta Med. Scand.* **112**, 84-89.
- Pratt, R., Sah, P. P. T., Dufrenoy, J., and Pickering, V. L. 1948. *Proc. Natl.*
Acad. Sci. U.S. **34**, 323-328.
- Professional Service Schools, Medical Department, Washington, D. C. U.S.A., *Bull.*
U.S. Army Med. Dept. **5**, 265 (1946).
- Quick, A. J. 1943. *Am. J. Physiol.* **140**, 212-220.
- Quick, A. J. 1945. *J. Am. Med. Assoc.* **126**, 1167.
- Quick, A. J. 1946. *J. Lab. Clin. Med.* **31**, 79-84.
- Quick, A. J., and Stefanini, M. 1948. *J. Biol. Chem.* **175**, 945-952.
- Rapoport, S., Wing, M., and Guest, K. G. M. 1943. *Proc. Soc. Exptl. Biol. Med.*
53, 40.
- Richert, Dan A. 1944. *J. Biol. Chem.* **154**, 1-8.
- Riegel, Byron. 1940. *Ergeb. Physiol. biol. Chem. exptl. Pharmacol.* **43**, 133-173.
- Rigdon, R. H., and Varnadoe, N. B. 1947. *J. Lab. Clin. Med.* **32**, 532.
- Rodahl, K., and Moore, T. 1943. *Biochem. J.* **37**, 166-168.
- Roderick, L. M. 1931. *Am. J. Physiol.* **96**, 413-425.
- Rosenberg, H. R. 1942. Chemistry and Physiology of the Vitamins. Interscience,
New York.
- Rosin, J., Rosenblum, H., and Mack, R. 1941. *Am. J. Pharm.* **113**, 434.
- Schmidt, Th., and Büsing, K. H. 1942. *Klin. Wochschr.* **21**, 411-415.
- Schoen, K. 1945. *J. Am. Pharm. Assoc., Sci. Ed.* **34**, 247.
- Schulek, E., and Rózsa, P. 1941a. *Magyar Chem. Folyóirat* **47**, 75-85.
- Schulek, E., and Rózsa, P. 1941b. *Chem. Abstracts* **35**, 7994.
- Schulek, E., and Rózsa, P. 1941c. *Mikrochemie ver. Mikrochim. Acta* **29**, 178-193.
- Schulek, E., and Rózsa, P. 1941d. *Z. anal. Chem.* **121**, 258.
- Schulek, E., and Rózsa, P. 1942. *Ber.* **75**, 1548-1557.
- Schwarz, H., and Ziegler, W. M. 1944. *Proc. Soc. Exptl. Biol. Med.* **55**, 160-164.
- Scudi, J. V. 1941. *Am. J. Physiol.* **133**, 440-441.
- Scudi, J. V. 1942. *Proc. Soc. Exptl. Biol. Med.* **50**, 16-17.
- Scudi, J. V., and Buhs, R. P. 1941. *J. Biol. Chem.* **141**, 451-464.
- Scudi, J. V., and Buhs, R. P. 1942a. *J. Biol. Chem.* **143**, 665-669.
- Scudi, J. V., and Buhs, R. P. 1942b. *J. Biol. Chem.* **144**, 599-606.
- Seegers, W. H., Loomis, E. C., and Vandenbelt, J. M. 1945. *Arch. Biochem.* **6**,
85-95.
- Seeler, A. O., Mushett, C. W., Graessle, O., and Silver, R. H. 1944. *J. Pharmacol.*
Exptl. Therap. **82**, 357.
- Sells, R. L., Walker, S. A., and Owen, C. A. 1941. *Proc. Soc. Exptl. Biol. Med.* **47**,
441.

- Shapiro, S., Redish, M. N., and Campbell, H. A. 1943. *Proc. Soc. Exptl. Biol. Med.* **53**, 251.
- Sheely, R. F. 1941. *J. Am. Med. Assoc.* **117**, 1603.
- Shemiakin, M. M., Schukina, L. A., and Shvezov, J. B. 1943. *Compt. rend. acad. sci. URSS* **65**, 2164-2167.
- Smith, C. C. 1947. *Proc. Soc. Exptl. Biol. Med.* **64**, 45-47.
- Smith, C. C., Fradkin, R., and Lackey, M. D. 1946. *Proc. Soc. Exptl. Biol. Med.* **61**, 398-403.
- Sproul, E., and Sanders, E. K. 1941. *Am. J. Physiol.* **135**, 137-148.
- Stein, H. B. 1944. *S. African J. Med. Sci.* **9**, 111-124.
- Stoppelman, Marie. 1942. *Acta Med. Scand.* **111**, 408-413.
- Summerson, H. 1943. *Federation Proc.* **2**, 72.
- Tarbell, D. S., Fukushima, D. K., and Dam, H. 1945. *J. Am. Chem. Soc.* **67**, 197-199.
- Tastaldi, H. 1945. *Rev. quim. e farm. Rio de Janeiro* **10**, 9-16.
- Tishler, M., and Sampson, W. L. 1948. *Proc. Soc. Exptl. Biol. Med.* **68**, 136-137.
- Trenner, N. R., and Bacher, F. A. 1941. *J. Biol. Chem.* **137**, 745-755.
- Tronsberg, G. 1944. *Nord. Med.* **21**, 13-14.
- Vennndt, H., and Plum, P. 1942. *Acta Med. Scand.* **111**, 396-407.
- Vonesch, E. E. 1941. *Anales farm. y bioquim. Buenos Aires* **12**, 109-116.
- Vonesch, E. E. 1942a. *Chem. Abstracts* **36**, 2500.
- Vonesch, E. E. 1942b. *Rev. farm. Buenos Aires* **84**, 115-121.
- Vonesch, E. E. 1942c. *Chem. Abstracts* **36**, 4841.
- Vonesch, E. E. 1943. *J. Am. Pharm. Assoc., Sci. Ed., Pharm. Abstracts* **32**, e 92 e.
- Wakim, K. G., Chen, K. K., and Gatch, W. W. 1943. *Surg. Gynecol. Obstet.* **76**, 323.
- Walker, S. E., Eylenburg, E., and Moore, T. 1947. *Biochem. J.* **41**, 575-580.
- Ware, A. G., Guest, M. M., and Seegers, W. H. 1947. *J. Biol. Chem.* **169**, 231-232.
- Wheatley, D. P. 1947. *Brit. Med. J.* **1947**, II, 689.
- Woolley, D. W. 1945. *J. Biol. Chem.* **159**, 59-66.
- Woolley, D. W. 1947. *Physiol. Rev.* **27**, 308-333.
- Woolley, D. W., and McCarter, J. R. 1940. *Proc. Soc. Exptl. Biol. Med.* **45**, 357-360.
- Zakharova, M. P., and Devyatnin, V. A. 1944. *Biokhimiya* **9**, 256.

Nutritional Requirements of the Cotton Rat and Hamster*

By B. S. SCHWEIGERT

*Division of Biochemistry and Nutrition, American Meat Institute Foundation,
University of Chicago, Chicago, Illinois.*

CONTENTS

	<i>Page</i>
I. Introduction	55
II. Nutritional Requirements of the Cotton Rat.....	56
1. General Considerations.....	56
2. Vitamin Requirements.....	57
3. Miscellaneous Growth Studies.....	60
4. Reproduction and Lactation.....	60
III. Nutritional Requirements of the Hamster.....	61
1. General Considerations.....	61
2. Vitamin Requirements.....	62
3. Reproduction and Lactation.....	64
IV. General Comments.....	64
V. Summary.....	65
References.....	66

I. INTRODUCTION

In the course of the development of the science of nutrition, it became readily apparent that the dietary requirements of animals varied for different species. The use of a number of different species therefore, greatly facilitated the discovering of nutritional factors, particularly the B vitamins, some of which are needed as dietary factors by one species but not by others. These studies were confined largely to the mouse, rat, chick, dog, monkey, and guinea pig. In recent years considerable interest has developed in studying the nutritional requirements of two other mammalian species, the cotton rat and the hamster. These animals have been widely used in virus studies and more recently in studies on the influence of diet on the incidence and extent of dental caries. Undoubtedly, the importance of these observations has prompted interest in studies on nutritional requirements.

* A portion of this material was presented at the Symposium on "Vitamin Requirements of Laboratory Animals," Division of Agricultural and Food Chemistry of the American Chemical Society, September, 1946.

II. NUTRITIONAL REQUIREMENTS OF THE COTTON RAT

1. General Considerations

The cotton rat is more excitable than the white rat and certain precautions are necessary in the care of these animals, particularly in the care and management of the stock colony. The breeding females are usually housed in individual cages and the young are disturbed as little as possible during the first few days of life. The gestation period is about 28 days and the litter size varies from 3 to 11. Further information on typical equipment and management procedures has been reported by Meyer and Marsh (1943), Meyer (1942), McIntire, *et al.* (1944) and Meyer and Meyer (1944).

Several studies have been made demonstrating the susceptibility of the cotton rat (*Sigmodon hispidus hispidus* and *Sigmodon hispidus littoralis*) to St. Louis encephalitis, Lansing strain of poliomyelitis, and to diphtheria toxin (Armstrong, 1939; 1940; Jungeblut, 1940). Other studies in which the levels of certain vitamins were varied (Weaver 1945, 1946) failed to reveal any relationship in the level of vitamins fed and the susceptibility to poliomyelitis virus.

Following the demonstrations of the importance of the cotton rat in virus studies, investigations were initiated to determine its dietary requirements, particularly of the B vitamins. Most of the work has been done with *Sigmodon hispidus hispidus*, consequently subsequent work to be reviewed refers to this species unless otherwise indicated. In the earlier work (McIntire *et al.*, 1944) a purified ration was devised for studies on the B vitamin requirements. In subsequent studies (Schweigert, *et al.*, 1945; Schweigert, 1947) the composition of the purified ration has been modified somewhat, specifically, the casein level has been increased from 18 to 24% and biotin and pteroylglutamic acid, which have recently become available, have been added. The composition of this purified ration is shown in Table I. For growth studies, the young were weaned at 15-20 days of age at an initial weight of 15-25 g.

Animals grow well when fed the purified diet indicated; however, the rate of growth is increased when liver concentrates are included in the ration (McIntire *et al.*, 1944; Schweigert *et al.*, 1945). Since these animals have not been inbred extensively, particular attention must be given to equal distribution of the young from each litter among the different dietary treatments in each experimental series. The growth rates of the males are more rapid than the females and should be taken into account in comparing the results obtained, particularly if equal numbers of females and males are not included in each group (Schweigert *et al.*, 1945; Anderson *et al.*, 1948).

TABLE I

Composition of Purified Ration Suitable for Growth Studies with the Cotton Rat

Basal components	Per cent	Vitamins	Mg./100 g.
Purified casein.....	24	Thiamine hydrochloride	0.25
Sucrose.....	67	Riboflavin	0.30
Salt mixture ¹	4	Pyridoxine hydrochloride	0.25
Corn oil (Mazola).....	4.7	Ca pantothenate	2.0
Fortified cod liver oil ²	0.3	Niacin	2.5
		Choline chloride	100.
		Pteroylglutamic acid	0.2
		Biotin	0.01
		<i>p</i> -Aminobenzoic acid	30.
		Inositol	100.

¹ Hegsted *et al.* (1941).² Nopco XX (3000 I.U. of Vitamin A and 400 I.U. of Vitamin D/g.).

2. Vitamin Requirements

The qualitative requirements for thiamine, riboflavin, pyridoxine (vitamin B₆), Ca pantothenate, niacin, biotin, choline, inositol, *p*-aminobenzoic acid, and pteroylglutamic acid (folic acid) have been determined, as well as information on the quantitative requirements for thiamine, riboflavin, pyridoxine and Ca pantothenate (McIntire *et al.*, 1944; Schweigert, 1947). A summary of these results and for the white rat is shown in Table II. The symptoms of the deficiency for thiamine, riboflavin, pantothenic acid and vitamin B₆ were similar to those observed for the white rat. It is also of interest that the amounts of these vitamins required for optimum growth of the cotton rat are similar to those required by the white rat. It should be pointed out that these requirements are tabulated for studies conducted with diets of similar composition, in that variants in the diet are known to influence the dietary requirements. The results presented here, however, are useful for comparative purposes.

It will be noted that the qualitative requirements are similar, with the exception of inositol. Also, the response noted when comparable diets are used is greater with the addition of niacin or liver concentrates for the cotton rat than for the white rat. The dietary dispensability indicated for several of the vitamins is based on observations that the addition of the factor to the diet does not increase the rate of growth.

In Table III detailed results obtained with inositol, choline, and liver extracts are presented. It will be noted that the omission of inositol resulted in a slow rate of growth, while the omission of choline resulted in a less marked retardation in growth as compared to the

TABLE II
B Vitamin Requirements for the Cotton Rat and White Rat for Growth
 (Mg./100 g. ration)

Vitamin	Quantitative	
	Cotton rat	White rat
Thiamine.....	.15	.08-.15
Riboflavin.....	.08 > < .30	.10-.15
Pyridoxine.....	.10	.08-.10
Ca pantothenate.....	.80	.80
	Qualitative	
Niacin ¹	±	±
Biotin.....	—	—
Pteroylglutamic acid.....	—	—
Inositol.....	+	—
Choline.....	+	+
p-Aminobenzoic acid.....	—	—
Unknown factors.....	+	+

¹ The response to niacin supplementation is dependent on the tryptophan content of the diet (see text).

TABLE III
Effect of Supplements of Inositol, Choline, and Liver Extract on the Growth of Cotton Rats
 (Six weeks growth period used)

Dietary regimen ¹	No. of animals	Grams gain per week
Inositol basal.....	14	2.9
Basal + 0.1% inositol.....	6	5.9
Basal + 0.1% inositol + .03% p-aminobenzoic acid.....	34	5.6
Choline basal.....	14 ²	6.7
Basal + 0.1% choline.....	12	8.0
Purified diet.....	32	6.9
Purified diet + 4% liver extract ³	31	9.0
Dog food stock ration.....	13	9.0

¹ The diets described as basal + 0.1% inositol, basal + 0.1% choline, and purified diet contain a complete vitamin supplement and are identical or similar to the diet described in Table I.

² Five of the animals died prior to 4 weeks. The results for choline were obtained at the end of 4 weeks on experiment.

³ A water soluble concentrate prepared from dried, defatted liver; Wilson Laboratories, Chicago, Illinois.

respective control groups. In later work, less striking differences were obtained in the rate of growth when inositol was omitted. Nevertheless, it does indicate that the requirement for inositol is different than for the white rat.

Recent studies with several species of animals, including the cotton rat, have shown that tryptophan can be converted to niacin. In brief, if sufficient tryptophan is provided either as protein or as the free amino acid, the apparent dietary requirements for niacin can not be demonstrated and an increase in the excretion of niacin derivatives occurs (Schweigert *et al.*, 1947; Schweigert and Pearson, 1948). A summary of the influence of the level of casein on the response to niacin supplementation is given in Table IV. It will be noted that when less than 24% casein is included in the diet and when a protein deficient in tryptophan (gelatin) is fed, that an increased growth performance and survival results when niacin is included in the diet. It is concluded, therefore, that the cotton rat, like the white rat, can utilize tryptophan to form niacin provided that sufficient tryptophan is present in the diet over that needed for protein synthesis.

TABLE IV

Effect of Level of Casein (Tryptophan) on the Dietary Requirement of the Cotton Rat for Niacin

Dietary regimen ¹	Growth rate (g./week)		Mortality of unsupplemented group
	Without niacin	With niacin ²	
12% Casein.....	1.7	5.6	5 out of 10 dead at 4 weeks
18% Casein.....	5.7	7.1	4 out of 16 dead at 6 weeks
24% Casein.....	8.7	8.0	None out of 7 dead at 6 weeks
12% Casein and 12% gelatin.....	3.0	5.6	2 out of 6 dead at 4 weeks

¹ These diets are modifications of the diet described in Table I with the casein content varied at the expense of sucrose and niacin either omitted or added at the level indicated.

² 1.0 mg. niacin added per 100 g. of ration.

In earlier work (McIntire *et al.*, 1944) 18% casein was used and, in accord with the work summarized here, a growth response was observed with niacin supplementation. It is apparent from subsequent work, however, that although the rate of growth of the white rat is increased only slightly when an 18% casein diet is fed, that the requirements for niacin for the cotton rat and white rat are qualitatively similar.

From the evidence presented in Table III, and from other data

(McIntire *et al.*, 1944; Schweigert *et al.*, 1945) it is clear that a marked growth response is observed when the purified diets are supplemented with liver extract, a good source of unknown factors. The magnitude of the response noted with the addition of liver extract is much greater than is obtained with the white rat. Although some preliminary studies have been done to fractionate the liver extract (Schweigert *et al.*, 1945) progress has been very slow due to the heterogeneity of the stock animals and the limited number of animals available.

The cotton rat does not require vitamin C for growth (Clark and Jungeblut, 1940) but does develop diarrhea and hemorrhages of the gingivae when 10% of glucoascorbic acid, an antimetabolite of ascorbic acid, is included in the diet (Woolley and Krampitz, 1943). These effects were noted in 5 days; however, no effects were noted when 2% glucoascorbic acid was fed for a period of 10 days. Information is not available on the requirements of the cotton rat for minerals or for the fat-soluble vitamins.

3. Miscellaneous Growth Studies

A high incidence and extent of carious lesions occur in the molars of cotton rats fed diets similar to the purified diet listed in Table I (Shaw *et al.*, 1944a and b; Schweigert *et al.*, 1945; Schweigert, Shaw *et al.*, 1946; Schweigert, Potts *et al.*, 1946; Anderson *et al.*, 1947, 1948). While few or no carious lesions develop in the white rat when such diets are fed, the cotton rat will develop from 20 to 40 cavities after 14 weeks on experiment. It is of particular interest that these diets permit an excellent rate of growth. When fermentable carbohydrates are fed the caries incidence is high; the incidence can be reduced by feeding starch or dextrin as the carbohydrate, by feeding a high-fat diet, milk as the sole diet, and other changes in the dietary treatment. These observations offer an excellent opportunity to study experimentally dietary factors that influence tooth decay.

It is of interest that the growth rate and survival is greatly reduced when starch or finely ground dextrin is fed as the carbohydrate. The animals scatter the food and fail to thrive. These results are in contrast to observations made with the white rat.

A high incidence of trichobezoar has been observed when purified diets were fed to cotton rats (Howell *et al.*, 1948) which could be prevented by including 10% cellulose in the diet.

4. Reproduction and Lactation

A few studies have been made on the influence of diet on reproduction and lactation performance (Schweigert, 1947, 1948). Since these

animals are very excitable and the mortality is high when they are mated as adults, the difficulties inherent in these studies are much greater than with the white rat. Some improvement can be obtained by placing the male and one or two females in a cage at weaning age. This technique, therefore, involves the influence of the diet on the performance of the male as well as the female, since both receive the same diet from an early age. Quite frequently pairs fail to produce litters even when fed stock rations, while others will raise excellent litters at regular monthly intervals. The reasons for this variation cannot be readily explained.

Some results obtained in preliminary studies are summarized in Table V. The stock pairs are disturbed as little as possible and one cannot readily obtain complete records on the performance. Nevertheless, the results indicate that fewer pairs produce young when purified rations are fed and, for the 8 months' period summarized, fewer pairs

TABLE V

Effect of Diet Composition on the Reproduction of the Cotton Rat

Dietary regimen	No. pairs producing young	No. litters produced (total)	No. young per litter (average)
Stock ration ¹	3 out of 4	21	4.5
Purified rations ²	4 out of 16	5	2.0
Purified rations + 4% liver extract.....	5 out of 7	16	2.6

¹ Rockland rat pellets, complete.

² A modification of the purified ration indicated in Table I. The casein level was increased to 30% at the expense of the sucrose, and the amount of vitamin A and D concentrate and the B vitamins was doubled with the exceptions of niacin, choline, inositol, and *p*-aminobenzoic acid.

produce successive litters. An improvement in the performance was obtained when liver extract was added, but the average size of the litters was considerably below that obtained for animals fed stock ration. The weights of the young at weaning were similar for all groups. These results indicate that although reproduction can be obtained with the use of a purified diet, more dietary factors are needed for optimum reproduction and lactation. It is recognized that the purified diets used favor the development of dental caries, and this may have a complicating effect in studies of this type. The animals eat readily, however, and reach a mature weight approximating that obtained when stock ration was fed.

III. NUTRITIONAL REQUIREMENTS OF THE HAMSTER

1. General Considerations

The Syrian or golden hamster (*Cricetus auratus*), like the cotton rat, has been an extremely valuable animal for public health work in that

it is susceptible to viruses and tubercle bacilli infections (Lennette, 1941; Broun *et al.*, 1941; Wheeler and Nungester, 1942; and Griffith, 1939). The incidence and extent of tooth decay are much higher in the hamster than in the white rat, but lower than in the cotton rat (Arnold, 1942; Keyes, 1946).

Difficulties in working with these animals are similar to those for the cotton rat. Descriptions of the care and management of colonies have been reported (Laidlaw, 1939; Hamilton and Hogan, 1944). The rate of growth and final weight of the hamster are somewhat less than that of the cotton rat. The gestation period is 16 days and the litter size ranges from 3 to 8. A purified ration has been devised for the hamster which supports growth rates comparable to those obtained when stock rations are fed (Hamilton and Hogan, 1944). The composition of this ration is given in Table VI. The cellular and chemical composition of the blood have been studied (Rose, *et al.*, 1946).

TABLE VI
Composition of Purified Ration Suitable for Growth Studies with the Hamster

Basal components	Per cent	Vitamins	Mg./100 g.
Purified casein.....	20	Thiamine hydrochloride	0.8
Cerelose ¹	65	Riboflavin	1.6
Lard.....	7	Pyridoxine hydrochloride	1.2
Cellulose.....	3	Ca pantothenate	1.0
Salt mixture ²	4	Niacin	5.0
Vitamin A-D mixture ³	1	<i>p</i> -Aminobenzoic acid	100
		Inositol	250
		Choline chloride	400
		2-Methyl-1, 4-naphthoquinone	3.0
		α -tocopherol	2.5

¹ Glucose monohydrate.

² Detailed composition given in a paper by Hamilton and Hogan (1944).

³ A mixture of oleum percomorphum and lard which supplied 1200 I.U. of vitamin A and 170 I.U. of vitamin D per g.

2. Vitamin Requirements

With the use of basal rations similar to that described in Table VI, Routh and Houchin (1942), Cooperman, *et al.* (1943), and Hamilton and Hogan (1944), have studied the qualitative requirements of the hamster for various vitamins. Their results are summarized in Table VII.

The omission of thiamine, riboflavin, pantothenic acid, or pyridoxine from the diet resulted in the production of deficiency symptoms similar to those observed in the white rat (Routh and Houchin, 1942; Hamilton and Hogan, 1944).

All three groups have studied the requirement for niacin. Routh

and Houchin (1942) reported that a marked weight loss, alopecia, and death occurred when niacin was omitted from the diet and these effects could be eliminated by the administration of 100 γ of niacin/day. The other investigators reported no deleterious effects when this vitamin was omitted. Cooperman *et al.* (1943) and Hamilton and Hogan (1944) used 18 and 20% casein diets, respectively, and from data obtained with other animals, it would appear that these amounts of casein should be approximately enough to eliminate the need for added niacin. Unfortunately,

TABLE VII
Qualitative Vitamin Requirements for the Growth of Hamsters

Vitamin	Qualitative requirement	Remarks on the deficiency and reference
Thiamine.....	+	Polyneuritis in 12 days ^{1,2}
Riboflavin.....	+	Death in 20 days ^{1,2}
Calcium pantothenate.....	+	Death in 20 days ^{1,2}
Pyridoxine (vitamin B ₆).....	+	Death in 24 days ^{1,2}
Niacin.....	±	Slow growth ¹ No effect ^{2,3}
Biotin.....	±	Slow growth ³ No effect ²
<i>p</i> -Aminobenzoic acid.....	—	No effect ²
Inositol.....	—	No effect ²
Choline.....	—	No effect ²
Vitamin K.....	+	Slow rate of growth, hemorrhage ²
Vitamin E.....	+	Collapse and death in 4-18 weeks ^{2,5}
Vitamin C.....	—	No effect ^{3,4}

¹ Routh and Houchin (1942).

² Hamilton and Hogan (1944).

³ Cooperman, *et al.* (1943).

⁴ Clausen and Clark (1943).

⁵ Houchin (1942a).

the level of casein used by Routh and Houchin was not given.¹ It seems likely that this factor may explain at least in part the discrepancies reported. Cooperman and associates attributed the difference to the fact that Routh and Houchin's work was complicated with a biotin deficiency. Later work by Hamilton and Hogan, however, indicated that neither niacin nor biotin was needed for optimum growth. Likewise, the latter workers observed that choline, inositol, and *p*-aminobenzoic acid were not required. Cooperman *et al.* reported better growth when the latter two substances were fed. As more work is done with the

¹ Dr. Routh (private communication) indicated that 20% of purified casein was used in these studies and that in later experiments a growth response with niacin supplementation was not consistently obtained.

hamster and more information is accumulated on the interrelationships of these factors, explanations for these apparent differences in results may be forthcoming. The role of pteroylglutamic acid (folic acid) is not known since it was not available when these studies were carried out.

The need for vitamins E and K during the growth period has been shown by Hamilton and Hogan (1944). The need for vitamin K by the hamster is of particular interest since the white rat does not require a dietary source due to the fact that sufficient amounts of this factor are synthesized by intestinal microorganisms. A somewhat slower rate of growth and hemorrhage occurred when vitamin K deficient diets were ingested. The animals fed vitamin E deficient diets collapsed and died in 4 to 18 weeks. If the animals were given α -tocopherol shortly after collapse they exhibited a rapid recovery. Similar results with vitamin E deficient diets have been reported by Houchin (1942a). In this and other studies changes in the oxygen consumption, chloride and creatine content of the tissues have been determined (Houchin, 1942b; Houchin and Mattill, 1942a and b).

The hamster, like the cotton rat and white rat, does not require vitamin C (Clausen and Clark, 1943; Cooperman *et al.*, 1943).

3. *Reproduction and Lactation*

Hamilton and Hogan also studied the requirements of the hamster for reproduction and lactation, and found that niacin, choline, and inositol were helpful although they could not demonstrate a need for these vitamins during the growth period. Further work was indicated to establish the essentiality of biotin or *p*-aminobenzoic acid. Only fair results were obtained when the following B vitamins were included: thiamine, riboflavin, vitamin B₆, niacin, pantothenic acid, choline, biotin, inositol, *p*-aminobenzoic acid. It is, therefore, apparent that the requirements for satisfactory reproduction are not completely known. Some of their results are shown in Table VIII. The results obtained with inositol-deficient diets are particularly striking. It is possible that those obtained on reproduction with the purified diets may be improved somewhat by increasing the level of protein above 20%, or by the inclusion of pteroylglutamic acid. The nature of the factors involved, as in the case of the cotton rat, are not known.

IV. GENERAL COMMENTS

It is of particular interest to accumulate knowledge on the nutritive requirements of the cotton rat and hamster, since these animals are considered to be less susceptible to infection than the guinea pig, but much more susceptible than the laboratory white rat. The possible

TABLE VIII

Effect of the Composition of the Diet on the Reproduction and Lactation Performances of the Hamster

Description of diet ¹	No. of litters	No. of young per litter (average)	No. of litters weaned	Comments
Stock.....	38	5.3	31	Failed to produce successive litters, weaning weights equivalent to group fed stock ration
Purified.....	5	5.4	5	
Biotin deficient.....	19	3.7	17	Somewhat lower weaning weights than for the group receiving biotin
Choline deficient.....	12	4.0	4	Low weaning weights
Niacin deficient.....	7	2.8	4	Low weaning weights
Inositol deficient.....	25	...	1	Several females died during parturition, many young born dead or failed to survive the 1st week of life

¹ The purified diet is the same as that described in Table VI and the vitamin deficient diets were prepared by omitting the appropriate vitamin from this diet.

relationship between their comparative nutritional requirements and their susceptibility to viruses and dental caries is a matter of speculation. With the information on the good growth performances obtained with the purified rations listed in Tables I and VI and partial success with reproduction using similar rations, studies on virus susceptibility, etc., can be made with the use of diets with defined composition. Much more work is needed, however, in expanding the knowledge now available on the requirements of the cotton rat and hamster not only for growth, but for reproduction and lactation as well.

V. SUMMARY

Satisfactory growth of the cotton rat and hamster has been obtained with the use of purified diets.

The quantitative requirements of the cotton rat and hamster for thiamine, riboflavin, pyridoxine (vitamin B₆), and pantothenic acid are similar to those of the white rat. Vitamin C is not required by either species.

The cotton rat grows at a more rapid rate when inositol is included in the diet. The response to niacin supplementation is influenced by

the protein (tryptophan) intake. Biotin, pteroylglutamic acid, and *p*-aminobenzoic acid are not needed in the diets for the normal growth of the cotton rat. Additional factors needed for growth and reproduction are present in liver extract.

For the hamster, the evidence is not conclusive on the essential nature of dietary sources of niacin, choline, biotin, inositol, and *p*-aminobenzoic acid for growth. Of particular importance is the demonstration that the hamster requires a dietary source of vitamin K.

Preliminary studies on the reproduction and lactation performances of the cotton rat and hamster when fed various purified diets have been presented.

REFERENCES

- Anderson, E. P., Smith, J. K., Elvehjem, C. A., and Phillips, P. H. 1947. *Proc. Soc. Exptl. Biol. Med.* **66**, 67-69.
- Anderson, E. P., Smith, J. K., Elvehjem, C. A., and Phillips, P. H. 1948. *J. Nutrition* **35**, 371-377.
- Armstrong, C. 1939. *U. S. Pub. Health Repts.* **55**, 1719-1721.
- Armstrong, C. 1940. *J. Bact.* **39**, 63.
- Arnold, F. A. 1942. *U. S. Pub. Health Repts.* **57**, 1599-1604.
- Broun, G. O., Muether, R. O., Mezera, R. A., and Le Gier, M. 1941. *Proc. Soc. Exptl. Biol. Med.* **46**, 601-603.
- Clark, A. R., and Jungeblut, C. W. 1940. *J. Nutrition* **20**, 427-432.
- Clausen, D. F., and Clark, W. G. 1943. *Nature* **152**, 300-301.
- Cooperman, J. M., Waisman, H. A., and Elvehjem, C. A. 1943. *Proc. Soc. Exptl. Biol. Med.* **52**, 250-254.
- Griffith, A. S. 1939. *J. Hyg.* **39**, 154-160.
- Hamilton, J. W., and Hogan, A. G. 1944. *J. Nutrition* **27**, 213-224.
- Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B. 1941. *J. Biol. Chem.* **138**, 459-466.
- Houchin, O. B. 1942a. *Federation Proc.* **1**, 117-118.
- Houchin, O. B. 1942b. *J. Biol. Chem.* **146**, 313-321.
- Houchin, O. B., and Mattill, H. A. 1942a. *J. Biol. Chem.* **146**, 301-308.
- Houchin, O. B., and Mattill, H. A. 1942b. *Proc. Soc. Exptl. Biol. Med.* **50**, 216-218.
- Howell, S. R., Schlack, C. A., McCay, C. M., and Taylor, B. L. 1948. *Science* **107**, 424-425.
- Jungeblut, C. W. 1940. *Proc. Soc. Exptl. Biol. Med.* **43**, 479-486.
- Keyes, P. H. 1946. *J. Dental Research* **25**, 469-473.
- Laidlaw, P. 1939. *Intern. J. Leprosy* **7**, 513-516; 1941. *Biol. Abstracts* **15**, 344.
- Lennette, E. H. 1941. *Proc. Soc. Exptl. Biol. Med.* **47**, 178-181.
- McIntire, J. M., Schweigert, B. S., and Elvehjem, C. A. 1944. *J. Nutrition* **27**, 1-9.
- Meyer, B. J. 1942. Ph D. Thesis. University of Wisconsin.
- Meyer, B. J., and Meyer, R. K. 1944. *Endocrinology* **34**, 276-281.
- Meyer, D. B., and Marsh, M. 1943. *Am. J. Pub. Health* **33**, 697-700.
- Rose, C. L., Hannah, J. R., and Chen, K. K. 1946. *Proc. Indiana Acad. Sci.* **55**, 190-195.
- Routh, J. I., and Houchin, O. B. 1942. *Federation Proc.* **1**, 191-192.
- Schweigert, B. S. 1947. Unpublished data.

- Schweigert, B. S. 1948. *Proc. Soc. Exptl. Biol. Med.* **68**, 522-525.
- Schweigert, B. S., and Pearson, P. B. 1948. *J. Biol. Chem.* **172**, 485-493.
- Schweigert, B. S., Pearson, P. B., and Wilkening, M. C. 1947. *Arch. Biochem.* **12**, 139-145.
- Schweigert, B. S., Potts, E., Shaw, J. H., Zepplin, M., and Phillips, P. H. 1946. *J. Nutrition* **32**, 405-412.
- Schweigert, B. S., Shaw, J. H., Phillips, P. H., and Elvehjem, C. A. 1945. *J. Nutrition* **29**, 405-411.
- Schweigert, B. S., Shaw, J. H., Zepplin, M., and Elvehjem, C. A. 1946. *J. Nutrition* **31**, 439-447.
- Shaw, J. H., Schweigert, B. S., Elvehjem, C. A., and Phillips, P. H. 1944a. *J. Dental Research* **23**, 417-425.
- Shaw, J. H., Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., and Phillips, P. H. 1944b. *J. Nutrition* **28**, 333-345.
- Weaver, H. M. 1945. *Am. J. Diseases Children* **69**, 26-32.
- Weaver, H. M. 1946. *Am. J. Diseases Children* **72**, 6-16.
- Wheeler, A. H., and Nungester, W. J. 1942. *Science* **96**, 92-93.
- Woolley, D. W., and Krampitz, L. O. 1943. *J. Exptl. Med.* **78**, 333-339.

Vitamins as Pharmacologic Agents

BY HANS MOLITOR AND GLADYS A. EMERSON

Merck Institute for Therapeutic Research, Rahway, New Jersey

CONTENTS

	<i>Page</i>
I. Introduction.....	69
II. Water Soluble Vitamins.....	75
1. The B Complex.....	75
Thiamine.....	75
Niacin.....	77
Riboflavin.....	79
Pyridoxine.....	80
Pantothenic Acid.....	81
p-Aminobenzoic Acid.....	81
Biotin.....	83
Pteroylglutamic Acid.....	83
Choline.....	85
2. Other Water Soluble Vitamins.....	86
Ascorbic Acid.....	86
Vitamin P.....	88
III. Fat Soluble Vitamins.....	89
1. Vitamin A.....	89
2. Vitamin D.....	90
3. Vitamin E.....	91
4. Vitamin K.....	92
References.....	93

I. INTRODUCTION

“Experimental pharmacology, in its broadest meaning, deals with the response of living organisms to chemical agents; or expressed in different words, examines the behavior of the organism in a chemically altered environment.” This definition by Hans Horst Meyer, one of the founders of modern experimental pharmacology, clearly indicates the difficulties facing the reviewers of a topic as broad as “Vitamins as Pharmacologic Agents.” Their task is complicated by the fact that the scientific literature provides relatively little assistance in the form of previous articles along similar lines. Indeed, while the fields of vitamin chemistry, biochemistry, physiology, pathology and, of course, therapy have been covered ably and thoroughly, it seems that the only earlier review of this topic was that presented by one of us (Molitor) in 1942 at a

symposium of the American Pharmacological Society. This is unfortunate because the selection of individual papers for inclusion in such a review is by necessity arbitrary and depends upon the reviewer's personal interpretation of the term "pharmacology."

Obviously, it is impossible to include more than a small fraction of the many hundreds of papers dealing with one of the many aspects of the topic; and thus the imposition of arbitrary limitations becomes unavoidable.

If we adopt Hans Horst Meyer's definition of pharmacology, this treatise would describe the pharmacologic effects of vitamins in healthy as well as in diseased states. However, since the therapeutic use of vitamins in the correction of a specific deficiency has been repeatedly reviewed, we feel justified in limiting the discussion to the effect of vitamins on normal animals and in diseases other than avitaminoses. In such cases the doses required are generally far in excess of those therapeutically effective in specific vitamin deficiencies. Such effects are, for example, the curare-like paralysis in frogs (Demole, 1938a); or the excessive salivation, nausea, vomiting, and collapse produced in dogs by intravenous injection of 50 mg./kg. of thiamine (Molitor, 1942).

Another nonspecific effect of a vitamin is the vasodilator action of niacin (Sebrell and Butler, 1938; Loman *et al.*, 1941; Positano and Ruggieri, 1942). However, one would not be justified to regard this as a pharmacologic property of niacin, *the vitamin*, since this effect is not produced by niacinamide (Field and Robinson, 1940) which otherwise fully shares the specific vitamin effects of niacin. Similarly, 2-methyl-1,4-naphthoquinone may produce severe skin irritation in sensitive individuals (Page and Bercovitz, 1942). Nevertheless this property is common to many compounds to which this quinone is not related structurally.

Other purely toxic effects of vitamins are those of pyridoxine, which in excessive doses produces clonic-tonic convulsions (Unna, 1940); or of riboflavin, which upon repeated intravenous injection of large doses is precipitated in the glomeruli, thus mechanically interfering with urinary excretion (Unna and Greslin, 1942). None of these "toxic" effects, however, are of even remote practical significance since the doses necessary to produce them are far beyond those therapeutically employed.

Any vitamin may be expected to restore to normal, functional changes which result from the deficiency of that vitamin. It is not easy, however, to determine whether therapeutic results obtained by administration of a vitamin in diseases not following the pattern of a typical deficiency are ascribable to properties other than those necessary for the function as a vitamin.

For example, the therapeutic effects of thiamine in cardiac failure of advanced beriberi (Weiss and Wilkins, 1937) or of niacinamide in psychosis of pellagra (Frostig and Spies, 1940) are recognized as the correction of typical vitamin deficiency states. We are not, however, in a position to state whether the therapeutic activity of pyridoxine in radiation sickness is a specific *vitamin* effect (Maxfield *et al.*, 1943), since it is unknown whether intense radiation produces a state of acute pyridoxine deficiency. The effect of large doses of vitamin D in rheumatic arthritis (Wyatt *et al.*, 1936; Vrtiak and Lang, 1936) or of ascorbic acid in asthma (Hagiesco *et al.*, 1938; Diehl, 1937) and hay fever (Holmes and Alexander, 1942) may serve as similar examples, provided that the alleged value of vitamin therapy in these conditions withstands critical examination.

As mentioned before, the amount of a vitamin necessary to produce "non-specific" therapeutic effects, is usually much larger than that needed to correct a deficiency, although it is still well below the toxic range. In turn, the doses needed to cure a fully developed vitamin deficiency state greatly exceed those sufficient to maintain an organism in optimum nutritional condition.

The great difference in effective doses in normal and in pathologic conditions need not be surprising, since it is well known that the optimal condition for demonstration of a drug effect is a state of stress and unbalance. Antipyretics, cardiacs, antispasmodics, analgesics, etc. may serve as examples for this.

Certain effects of vitamins cannot be observed by simple inspection or application of the commonly used pharmacologic technics. Thus, Porter *et al.* (1947) have shown that in rats the only change produced by the administration of desoxypyridoxine (a vitamin analogue of pyridoxine) is an increase in the urinary excretion of xanthurenic acid. While a metabolic effect such as this cannot be termed pharmacologic, it might well indirectly alter the conditions required for the action of other drugs. Seeler (1945) observed that administration of pyridoxine to malaria infected birds abolished the curative effect of quinine or atabrine, although in the dose used pyridoxine failed to produce visible effects when injected in either normal or infected birds. Similarly, *p*-amino-benzoic acid inhibits in normal animals the effect of sulfa drugs; and vitamin K prevents the appearance of the hemorrhages following administration of sulfaquinoxaline (Seeler *et al.*, 1944a) or dicumarol (Davidson and MacDonald, 1943; Lucia and Aggeler, 1944).

On the basis of the foregoing considerations, we feel inclined to include in this review vitamin actions which are pharmacodynamic in character, regardless of whether they occur in normal or deficient animals; we shall omit, however, a discussion of those pharmacologic effects of

vitamins which constitute a restoration to normal of a typical vitamin deficiency.

The comparatively minute dose of a vitamin needed to cure a specific deficiency, contrasted to the much larger dose necessary to produce an effect in the normal animal or in one affected with a disease not caused by a vitamin deficiency, often permits a diagnosis on the principle "post hoc ergo propter hoc"; indeed, several pathologic conditions of hitherto unclear etiology have been elucidated by their dramatic response to small doses of vitamins. We have in mind particularly the neurologic and psychiatric disorders associated with deficiencies of the B complex, for example the Wernicke-encephalopathy (paralysis of the eye muscles, balance disturbance, and drowsiness, usually terminating in fatal coma) and the Korsakoff psychosis (delirium, confabulation, loss of memory without loss of consciousness). However, while many cases of this psychiatric disorder, as well as of delirium tremens, promptly respond to relatively large (10-50 mg.) doses of intravenously administered thiamine (Bowman *et al.*, 1939; Roch and Sciclounoff, 1938; Caldwell and Hardwick, 1944), there is considerable doubt whether a Korsakoff psychosis etiologically unrelated to thiamine deficiency (such as caused by head trauma or subarachnoid hemorrhage) is influenced by thiamine medication. Indeed, in such cases vitamin administration can serve diagnostic purposes.

An even more striking illustration of the diagnostic value of vitamin administration is the response to B complex vitamins in cases of so-called "subclinical" deficiency diseases. Mental symptoms such as irritability, inability to concentrate, depression, phobias, and anxieties, have been noted as some of the earliest signs of experimentally induced B complex deficiency (Jolliffe *et al.*, 1939; Williams *et al.*, 1940; Sebrell, 1943); prompt relief by vitamin administration may establish a diagnosis which otherwise would be difficult to make.

Another diagnostic use of a vitamin is that of vitamin K in cases of hepatopathy. Since the liver is the site of prothrombin formation Lord and Andrus (1941) have postulated that failure of vitamin K to correct hypoprothrombinemia in jaundiced patients is strong evidence for liver injury; and Turner *et al.* (1944) have successfully used the response to vitamin K as a prognostic criterion in patients suffering from acute hepatitis; and Unger and Shapiro (1948) have used a standard vitamin K tolerance test in estimating the prothrombin response to parenteral administration of large test doses of vitamin K. This test was found to be a sensitive indicator of hepatic disease.

The high specificity of vitamins can be demonstrated in experimentally produced pathologic conditions which closely resemble those

observed in vitamin deficiencies, but are unrelated to them etiologically. Molitor and Sampson (1937) placed a group of rats on a thiamine deficient diet, while pair-fed controls received a complete ration. In both groups, frequent records were taken of body weight, heart rate and sensitivity to the convulsant drug thujone. As the animals lost weight, both groups became more sensitive to the convulsant agent, but only the thiamine deficient group showed the typical bradycardia, which responded within less than 1 hour to an intravenous injection of thiamine.

Failure to respond to vitamin therapy does not justify the conclusion that the pathologic condition is not due to a vitamin deficiency. Factors such as interference with absorption, antagonistic effect of specific vitamin inhibitors, changed physiological conditions (lactation, pregnancy, strenuous work) or an unbalance in the composition of the diet may greatly affect the vitamin requirements. Thus, the average therapeutic dose of niacin or niacinamide in moderately severe cases of pellagra is 25 to 50 mg. by mouth three times daily; fully developed cases of pellagra psychosis, however, require such heroic doses as 100 mg. of niacin intravenously in hourly intervals, up to 1 gram daily (Jolliffe *et al.*, 1940). Similarly the average daily dose of thiamine in uncomplicated cases of thiamine deficiency is 5 to 10 mg. orally, but severe cases of beriberi heart-failure, may require the intravenous injection of 25 to 130 mg. daily (Weiss and Wilkins, 1937). One reason for administering to critically ill patients vitamins by the parenteral route and in doses far above those required for normal maintenance is the impaired absorption often found in deficiency diseases. Another is the belief that very large doses of a drug may exert a "mass effect" capable of turning the tide of a progressing pathologic condition when smaller doses would fail (Williams and Spies, 1938). Since the toxicity of vitamins as medicinal agents is extremely low, no objection would seem to exist against the use of unusually large doses, as long as the administration takes place under medical supervision.

One of the principal obstacles in establishing the cause-and-effect relationship in vitamin therapy is the difficulty of an early diagnosis and quantitative evaluation of vitamin deficiencies. Much progress has been made in the last few years, particularly since the introduction of sensitive and easily performed microdeterminations of the vitamin concentration in the blood, urine, and body tissues (Lowry and Bessey, 1944; Lowry *et al.*, 1945; Lowry and Bessey, 1946). Such methods, however, are not yet available for some of the most interesting vitamins, notably, pyridoxine. Furthermore, it must be borne in mind that the vitamin concentration in the blood is not necessarily an indicator for the state of vitamin saturation of the body as a whole, since the concentration in the

tissues may vastly differ from that in the circulating blood (Youmans *et al.*, 1941). Indeed, even the tissue concentration may vary with the function of the tissue. For example, thiamine is firmly retained in heart and brain at a time when it already has reached dangerously low levels in less vital organs (Ferrebee *et al.*, 1942).

A more indirect method of appraising the adequacy of the vitamin supply is to follow the urinary excretion of a vitamin after administration of a test dose (Abbasy *et al.*, 1935; Mason and Williams, 1942). The theory underlying this approach is that in an organism saturated with vitamin, most of the test dose is likely to be excreted, whereas a depleted organism will retain and store it. It is not possible, however, to estimate solely on this basis the requirements for a vitamin since the differences between the amounts administered and excreted vary with the size of the test dose. Thus, of 100 γ of pantothenic acid given to a rat, 7 γ were excreted (Silber and Unna, 1942); of 250 γ , only 67; and of 3000 γ , only 600. On the basis of these findings the pantothenic acid requirement might have been estimated as 93, 183 and 2400 γ , respectively, while on the basis of growth experiments it actually is about 100 γ . A similar observation has been made in children receiving varying doses of thiamine (Knott, 1936). Increasingly high retentions were found with higher levels: the optimum utilization being at about 100 γ /kg./day.

A still less satisfactory way to estimate the degree of vitamin saturation of the body is the performance of balance experiments and the determination of the amount of vitamins excreted in the feces. It is well known that many vitamins are synthesized by the intestinal bacteria and that this process is influenced not only by the composition of diet, but also by the action of drugs. A diet rich in fat appears to favor the biosynthesis of thiamine (Whipple and Church, 1935) while chemotherapeutic agents such as the sulfonamides and certain antibiotics interfere with biosynthesis (Black *et al.*, 1941; Emerson and Smith, 1945). It must be kept in mind, however, that the vitamins excreted in the feces would not necessarily be available to the organism. For this reason it would seem somewhat risky to judge the state of the body's vitamin stores solely from the outcome of urinary and fecal balance experiments.

As with many other drugs, the mode, rate and frequency of administration are of considerable influence on the pharmacologic and toxicologic effects of vitamins. Adverse reactions are least likely with peroral administration, and most likely with intravenous. This is due partly to the sudden, high drug concentration obtained by the latter mode of administration, partly to the possibility of side-reactions of an allergic (anaphylactic) nature. Thus, the only serious accidents reported with thiamine therapy occurred when it was injected parenterally (Mills,

1941); the signs and symptoms reported were those of an allergic reaction rather than of a specific pharmacodynamic drug effect. In animals, however, it has not been possible to produce such a sensitization. Dogs and guinea pigs injected daily for 10 consecutive days with 50 mg./kg. of thiamine (a dose sufficient to produce severe toxic reactions) failed to show signs of anaphylactic shock, when the animals were challenged after a treatment-free interval of from 10–60 days (Molitor and Seeler, unpublished).

It cannot be emphasized too strongly that experiments of the acute type, viz. single administration of very large doses, have but little value for the appraisal of the toxic properties of a vitamin. Since vitamin therapy requires, as a rule, repeated administration of small or medium doses over an extended period of time, chronic toxicity tests are indispensable for forming valid conclusions regarding the potential toxicity of a vitamin. Such tests should be conducted over the entire lifespan of the test animal and should preferably be continued over several generations (Perla, 1937; Sure, 1940).

Vitamins in general have a very low order of toxicity, the only exception to this being the vitamin D's. While some vitamins, such as riboflavin and pteroylglutamic acid produce certain lesions of the kidney, when administered in very large doses, this is due to mechanical rather than specific chemical factors and appears to be directly related to the low order of solubility of these compounds. Other toxic manifestations occasionally observed following the parenteral administration of vitamins seem largely to be of the type of an allergic reaction and can hardly be regarded as a specific toxic property of the particular vitamin.

II. WATER SOLUBLE VITAMINS

1. *The B Complex*

Thiamine. The lethal doses on intravenous injection of thiamine hydrochloride for mice, rats, rabbits, and dogs respectively are 125, 250, 300, and 350 mg./kg. of body weight (Molitor and Sampson, 1936); subcutaneously and perorally the fatal doses were six and forty times larger. Signs of toxicity are shock, twitching of the muscles, disturbed breathing, and finally, respiratory failure. The ratio for the therapeutic dose to the minimum toxic dose in monkeys was approximately 1:15,000 (Hecht and Weese, 1937). Rabbits appeared normal after the prolonged administration of 50 mg./kg. of thiamine intravenously daily. However, Haley and Flesher (1946) have recently reported that the intravenous injection of 200–300 mg. (total dose) of thiamine usually resulted in

collapse and/or death of rabbits. Recovery occurred in animals in which the injection was terminated before respiration ceased.

Large quantities of thiamine administered to rats daily throughout life resulted in a loss of maternal instinct and disturbance of lactation in succeeding generations (Perla, 1937; Sure, 1940; Molitor, 1942; Perla, 1939; Perla and Sandberg, 1939). Perla has reported that this effect can be counteracted by the addition of small quantities of manganese to the diet.

Injection of large doses of thiamine to frogs (Demole, 1938) produces a typical curare-like paralysis of the striated muscle, which can be counteracted by prostigmine. Unna and Pick (1944) have shown that thiamine antagonizes the effect of nicotine on isolated smooth muscle; the site of this action is probably the myoneural synapses and end plates at the myoneural junction.

Toxic manifestations of thiamine administrations in humans are rare and are most likely of allergic origin. Steinberg (1938) and Mills (1941) reported that herpes zoster was observed in 3 cases following intensive thiamine therapy. Other untoward reactions were nausea, epigastric fullness, and cramps, and symptoms resembling an overdosage of thyroxine (Eisenstadt, 1942; Leitner, 1943; and Stein and Morgenstern, 1944). Williams and Spies (1938) have stated that "because of the great difference between the toxic dose, and the absence of evidence for cumulative toxicity, vitamin B₁ is a remarkably safe therapeutic agent and may be used without concern in long extended treatment." These investigators have given normal subjects 500 mg. daily for a month without any objective toxic effects; and even severely ill patients tolerated the intravenous injection of 20 times the usual dose of thiamine. Jolliffe (1941) administered thiamine to more than 3,000 patients without untoward reactions. Bicknell and Prescott (1946), during a period of 7 years, did not encounter a single case of intolerance.

Although untoward reactions following thiamine administration have been observed it should be emphasized that cases of intolerance are comparatively rare and usually occur only with parenteral administration of the vitamin. For this reason it has been suggested by the editors of *Lancet* (1947) that thiamine hydrochloride should be administered only by mouth except in severe intestinal dysfunction and acute beriberi. If parenteral administration must be used, the risk of anaphylaxis should be borne in mind and doses of 50 to 100 mg. should be given very slowly.

Successful desensitization to thiamine has been accomplished by the subcutaneous injection of graded doses of thiamine (Mitrani, 1944).

Mention should be made of the use of thiamine in the treatment of conditions other than thiamine deficiency. Thiamine is an essential

factor in the transmission of nerve impulses; its activity in this respect being due to the inhibition of choline esterase formation and thereby augmentation of the acetylcholine effect on the nerve endings (Abderhalden and Abderhalden, 1938; Jackson and Wald, 1942). Pick and Unna (1945) reported that thiamine suppressed the electrical potentials in frog brains in doses representing $\frac{1}{10}$ to $\frac{1}{50}$ of those effective on the myoneural junction; while with curare and erythroidine the doses effective in decreasing the electrical activity of the brain were slightly larger than those which blocked transmission at the myoneural junction.

Many papers have appeared on the treatment of peripheral neuritis since the initial communication of Vorhaus, *et al.* (1935). Kalaja (1941) concluded, following the treatment of 51 cases of neuritis of varying etiology, that only the symptoms due to a deficiency in thiamine respond to thiamine therapy.

Likewise, the favorable influence of thiamine on the polyneuritis and peripheral neuritis of pregnancy may be ascribed to the existence of a dietary deficiency (Bingham, 1936; Herzberg, 1941).

The role of thiamine in the treatment of heart failure was appraised in an editorial appearing in the British Medical Journal (1944). It was considered doubtful if the vitamin had any therapeutic effect on the heart except in cases of beriberi and long-established alcoholism.

Other therapeutic claims for thiamine include its effectiveness in diabetes mellitus (Vorhaus *et al.*, 1935), gout (Vorhaus *et al.*, 1935), seasickness (Holmes, 1944), radiation sickness (Whitmore, 1943), acrodynia (pink disease) (Durand *et al.*, 1939), hyperthyroidism (Williams *et al.*, 1943). However, none of these claims could be upheld upon critical examination and the beneficial effects which unquestionably resulted from thiamine administration in many of these conditions could be ascribed to the correction of a coexisting state of thiamine deficiency.

Niacin. Early experiments by Hunt and Renshaw (1929) indicated that 20 mg. of niacin hydrochloride per kilogram of body weight was nontoxic in mice and was without effect on the blood pressure of the cat. Later Unna (1939) reported the acute toxicity (LD 50) of niacin in mice and rats as from 4–5 g./kg. by the subcutaneous and 5–7 g./kg. by the oral route of administration; niacinamide was about twice as toxic. Prolonged oral administration of large amounts (up to 2 g./kg. daily) of sodium salt of niacin to rats, chickens or dogs for periods up to 2 months failed to produce toxic signs or pathological changes. The sodium salt of niacin and niacinamide were without effect upon blood pressure and respiration of the cat when administered in doses up to 1 g./kg. However, the injection of niacin (0.5% solution, pH 3.3) even in small amounts (5 to 10 mg.) frequently raised the blood pressure by 10 to 20 mm. Hg.

Since HCl at the same pH gave a like result it is justifiable to ascribe this rise in blood pressure, first reported by McCrea (1938), to the acidity of the injected solution.

Brazda and Coulson (1946) reported that niacin and trigonelline, when administered subcutaneously to rats, showed such a low order of toxicity that accurate determination was impossible. Methylation decreased the toxicity of niacinamide but was without influence on that of niacin.

The vasodilating properties of niacin were observed in humans by Sebrell and Butler (1938), Sydenstricker *et al.* (1938), Spies *et al.* (1938). The administration of niacin to both normal subjects and to pellagrins, brought about a flushing of the face and neck and a sensation of heat, tingling, and itching. Sometimes slight dizziness, headache, and nausea were observed. The symptoms were only transitory, though disturbing to the patient. These side effects were not produced by niacinamide. Oral doses of 100 to 300 mg. or 20 to 25 mg. subcutaneously caused an increase in skin temperature but not in body temperature. Sodium, ammonium and monoethanolamine salts of niacin as well as its ethyl ester produced the same reaction. The vasodilator action was reversed by adrenaline.

The administration of niacinamide in high dosage to subjects saturated with niacin did not produce the peripheral vasodilation or other vascular symptoms which followed the injection of large doses of niacin.

In addition to its effect on the arterioles of the skin, the vasodilator action of niacin is also noted in cerebral and spinal vessels (Loman *et al.*, 1941).

Calder (1947), in experiments on the action of niacin on the isolated rabbit's heart, reported that the effect on the normal organ was insignificant: in myocardial failure, however, the compound produced a marked increase in amplitude, reversal of abnormal rhythms, and at times a considerable augmentation of coronary flow. Calder is of the opinion that the disturbances of myocardial action are due to depletion of the pyridine nucleotides, and that this is counteracted by the addition of niacin to the perfusion fluid.

The nonspecific vasodilating effect of niacin has been used in the treatment of a number of diseases in which the use of a vasodilator is indicated. Niacin therapy has proved efficacious in cases of Meniere's syndrome (Atkinson, 1941), perennial vasomotor rhinitis (Williams, 1946), idiopathic or hypertensive headaches, typical migraine and headache following spinal taps (Goldzieher and Popkin, 1946) and trigeminal neuralgia (Poser, 1942; Karl *et al.*, 1945).

The vasodilating property of niacin may be regarded as respon-

sible for its efficacy in the treatment of asthma (Melton, 1943; Maisel and Somkin, 1942), angina pectoris (Neuwahl, 1942), and chronic coronary insufficiency (Uhlmann, 1944). Miscellaneous uses of niacin have been reported such as in the treatment of chilblains (Calvert, 1946), Vincent's angina (Johnson, 1945), various types of dermatoses not associated with pellagra (Schmidt, 1945) and in allergic reactions to penicillin (Service, 1946).

Mention should be made of recent studies which have shown a definite relationship between the tryptophane intake and the niacin requirement of several species. Krehl *et al.* (1945a) reported that tryptophane counteracted the growth retarding effect of corn grits in niacin low rations in the rat; a similar finding was observed in the dog (Krehl *et al.*, 1945b).

Riboflavin. Early observations (Kuhn and Boulanger, 1936) indicated that riboflavin was nontoxic when administered intraperitoneally at a level of 340 mg./kg. to mice. Demole (1938b) reported that the rat tolerated a dose of riboflavin 5000 times greater than the therapeutic dose without showing any toxic signs. Riboflavin was nontoxic to fish, frogs, mice, rabbits, cats and dogs following single or repeated doses ranging from 1 to 50 mg./kg. of body weight. Unna and Greslin (1942) reported that the LD 50 of riboflavin following intraperitoneal administration was 560 mg. per kg. Death, due to mechanical impairment of kidney function by concretions, occurred in 2 to 5 days. Oral administration of riboflavin to rats (10 mg. per kg.) and to dogs (2 g./kg.) failed to produce any untoward effects as the low solubility of riboflavin prevented its absorption from the gastrointestinal tract in amounts sufficient to produce toxic changes. Daily administration of 10 mg. of riboflavin over periods of four months to rats and 25 mg./kg. to dogs was well tolerated. Rats receiving 10 mg. daily were raised through three generations without showing pathologic signs.

Pathological changes in the urinary tract of rats were observed following the intraperitoneal administration of excessively large doses (125-500 mg./kg.) of riboflavin (Antopol, 1942). A precipitate of the vitamin was found in the kidneys and the urinary tract. The pathologic effects consisted of obstructive and inflammatory kidney lesions with resultant uremia and a calcifying nephrosis.

Riboflavin has been clinically employed in the treatment of symptoms ascribable to a deficiency of this vitamin. Since, however, under certain conditions, such as exposure to bright light and eye strain, the requirement for this vitamin is apparently greatly increased, riboflavin therapy may prove successful even when the dietary intake appears to be completely adequate (Tisdall *et al.*, 1943).

C. B. Smith (1946) has reported the improvement of patients with simple and ophthalmic migraine, when 15 mg. of riboflavin daily was given for several months.

Since the initial observation of Kinoshita (1937) on the production of hepatomas in rats by *p*-dimethylaminoazobenzene, an attempt has been made to associate the state of nutrition with susceptibility to tumor formation (Nakahara *et al.*, 1939a; Nakahara *et al.*, 1939b; Kensler *et al.*, 1941). The administration of large amounts of riboflavin (10 mg., 3 times weekly) retarded the occurrence of pathologic changes in the liver produced by *p*-dimethylaminoazobenzene (Antopol and Unna, 1942). It is of interest to note that the concentration of riboflavin is lower in tumor than in normal tissue (Pollack *et al.*, 1942).

Pyridoxine. The toxicity of pyridoxine was investigated by Unna (1940) who found that the LD 50 of the hydrochloride in rats was 3.7 g./kg. following subcutaneous injection and 5.5 g./kg. on oral administration. Doses in excess of 1 g./kg. produced toxic manifestations in dogs, rabbits, and rats characterized by impairment of coordination, and tonic convulsions. The dose required to produce these signs was at least 1000 times greater than the therapeutic dose. Daily administration of 20 mg./kg. of pyridoxine to dogs and 25 mg./kg. to rats failed to produce toxic manifestations or pathological changes. Rats receiving 2.5 mg. daily were raised through three generations. Twenty milligrams per kilogram injected intravenously into cats was without effect upon blood pressure or respiration. Isolated organs (uterus and intestine) of rabbits were not influenced by pyridoxine in a concentration of 1:10,000.

The role of pyridoxine in human nutrition is unknown. This vitamin has been employed in the treatment of several types of muscular dystrophy with inconclusive results (Jolliffe, 1940; Meller, 1942; Rudesill and Weigand, 1941; and Spies *et al.*, 1940). Pyridoxine appears to give some promise in the therapy of acne vulgaris (Jolliffe *et al.*, 1942).

Recent investigations by Stoerk *et al.* (1947) and by Axelrod *et al.* (1947) indicate that pyridoxine may be involved in the development of immunity as rats deprived of this vitamin showed a striking impairment in antibody response.

The effectiveness of pyridoxine in the treatment of radiation sickness appears to be established. A large number of confirmatory reports (Reeves, 1946; Oppenheim and Lih, 1946; Wells and Popp, 1947) have appeared since the original paper by Maxfield *et al.* (1943). No rationale has been found for this action of pyridoxine since it is not known what, if any, changes occur in the blood and tissue levels during radiation therapy or whether the availability of pyridoxine is affected by such treatment.

Pyridoxine has been employed in nausea and vomiting from other causes including pregnancy and motion sickness without definite objective results. Molitor and Kuna (personal communication) investigated the effect of large parenteral doses of pyridoxine in dogs upon experimentally induced vomiting. The animals were maintained on a balanced diet, containing adequate amounts of pyridoxine and in addition were given pyridoxine up to 250 mg./kg. subcutaneously preceding, or simultaneously with, emetic doses of apomorphine. No changes were noted the frequency, severity, time of onset, and duration of vomiting. These findings indicate that pyridoxine is without effect upon the vomiting center.

Pantothenic Acid. The toxicity and pharmacology of pantothenic acid were investigated by Unna and Greslin (1940, 1941). As with the other vitamins of the B complex, the toxicity is low. The LD 50 following subcutaneous injection is 2.7 g./kg. in mice and 3.4 g./kg. in rats. The daily administration of calcium pantothenate to monkeys (1 g./monkey), dogs (50 mg./kg.) and rats (1 g./kg.) failed to produce any toxic manifestations or pathological changes. Instillation of 0.5 ml. of a 10% solution of pantothenic acid into the conjunctivae of rabbits produced no irritation. The subcutaneous injection of 1 ml. of a solution of the same concentration did not produce irritation, inflammation or abscesses in rabbits. Calcium pantothenate is likewise well tolerated in humans. Doses of 100 mg. were administered intravenously to normal persons without producing significant changes in blood pressure, pulse or respiration (Spies *et al.*, 1940).

Rats maintained on a pantothenic acid deficient diet are capable of developing heavier infections with *Trypanosoma lewisi* than normal rats (Taylor and Becker, 1948). While the omission of pantothenic acid from the diet of experimental animals produces a well defined pathological syndrome, the role of this vitamin in human nutrition has not been established.

p-Aminobenzoic Acid. *p*-Aminobenzoic acid is not a specific growth factor for the rat. It is, however, an essential metabolite for certain microorganisms. The deficiency syndrome produced in rats by the administration of certain sulfonamides in conjunction with purified diets can be negated by PABA (Black *et al.*, 1942) due possibly to stimulation of the synthesis of other vitamins by intestinal microorganisms. Briggs *et al.* (1943) have reported that PABA is not a specific growth factor for the chick but may indirectly influence the production of other vitamins.

Scott and Robbins (1942), in an investigation of the acute, oral toxicity of PABA found that the drug was better tolerated by rats than by mice or dogs. Doses in excess of 1.0 g./kg. in dogs resulted in the

death of some of the animals. Acute gastroenteritis and hemorrhages into the small intestine were observed. Acute necrosis of the liver was seen in dogs receiving 2.0 g./kg. or more. Rats tolerated doses of 1.4 g./kg. by mouth for about a month without inhibition of growth or pathological changes. No deaths occurred unless at least 6 g./kg. were fed daily for 3 successive days. Following intravenous injection of 4 g./kg., 30% of the animals died (Richards, 1942), the manifestations of toxicity being convulsions and respiratory paralysis. Young rats injected with 200–500 mg./kg. daily for 21 days grew at a slightly faster rate than did their controls. In rabbits the intravenous LD 50 was approximately 2 g./kg. High intakes of PABA (up to 2 g./kg. orally) have been tolerated by patients receiving the drug in the therapy of rickettsial infections. The chronic toxicity of PABA is low.

The blood pressure of dogs and cats was slightly raised by doses of 100 mg./kg. but this increase was dependent upon the rate of injection. Respiration was not affected. The oxygen consumption of rats was slightly but not consistently increased after intraperitoneal injection of 600 mg./kg.

PABA has a detoxicating action against massive doses of the various pentavalent arsenicals (Sandground, 1943). The protective effect was dramatically demonstrated within 24 hours following the intravenous administration of high doses of acetarsone. At a time when nearly all control rats were dead or *in extremis*, only a few animals receiving adequate amounts of PABA gave evidence of acetarsone intoxication. The drug appeared to be somewhat effective in the relief of central nervous system disturbances produced by arsenicals.

PABA in large doses is unquestionably effective in the treatment of rickettsial infections, both experimental (Snyder *et al.*, 1942; Greiff, *et al.*, 1944; Anigstein and Bader, 1945; Hamilton, 1945; Hamilton *et al.*, 1945; Snyder and Zarafonitis, 1945; Murray *et al.*, 1945) and clinical (Yeomans *et al.*, 1944; Rose *et al.*, 1945; Maroney *et al.*, 1946; P. K. Smith, 1946; Levy and Arnold, 1946; Hendricks and Peters, 1947; Ravenel, 1947a; Tierney, 1946; Tierney, 1947).

The suggested dose of PABA for the treatment of rickettsial disease is 2 g./kg./day (Ravenel, 1947b; Faust, 1946). This intake maintains a blood concentration of 30–60 mg./100 ml. Blood levels in excess of 80 mg./100 ml. may prove hazardous. Possible toxic reactions are acidosis, leukopenia, abdominal distention, and delirium. The dosage suggested by Ravenel is somewhat higher than that employed in the treatment of typhus infections by Yeomans *et al.* (1944) and Snyder *et al.* (1947).

PABA has been used by Dry (1946) and Dry *et al.* (1946), to raise the salicylate level in the blood of patients receiving salicylates in the treat-

ment of acute rheumatic fever. Rosenblum and Frazer (1947) observed that PABA per se was of value in reducing the temperature and relieving pain in rheumatic fever.

Biotin. Schmidt and Landy (1942) in a study of the pharmacological properties of biotin reported that the rapid intravenous injection of 500 γ of biotin did not influence the blood pressure, heart rate and respiration of the cat. Biotin in concentrations up to 1 part in 40,000 was without effect upon smooth muscle *in vitro*; perfusion of the frog heart *in situ* with 200 γ of biotin produced no significant alteration in amplitude, rate, or rhythm. Employing dosages as high as 5 mg. Crittenden (1948) found that biotin was devoid of specific pharmacodynamic properties; its order of toxicity was low. Mice receiving 1 mg. of biotin per os daily for a period of 60 days showed no untoward effects. Likewise, toxic signs were not observed in rats dosed orally for 10 successive days with 50 mg. of biotin.

The physiological role of biotin is unknown. Most species are capable of synthesizing biotin and a deficiency syndrome can be induced only by the incorporation of raw egg white, its active principle avidin, or one of the sulfonamides in the diet. The chick, however, is apparently unable to synthesize sufficient biotin to meet its needs.

The first experimental evidence indicating that biotin deficient chicks are less resistant to infection with *Plasmodium lophurae* than normal birds was presented by Trager (1943a,b). This observation was confirmed by Seeler *et al.* (1944b).

Susceptibility to *Trypanosoma lewisi* infection is greatly increased in biotin deficient animals (Caldwell and György, 1943). Furthermore, rats in the terminal stages of biotin deficiency were not protected from infection with *T. lewisi* by injection of hyperimmune serum in amounts that completely protected normal rats (Caldwell and György, 1947).

Biotin has been reported to have a procarcinogenic action in malnourished rats in which hepatic tumors were induced by *p*-dimethylaminoazobenzene (butter yellow). The protection afforded by casein and riboflavin when added to the ration appeared to be counteracted by biotin (du Vigueaud *et al.*, 1942). Tumor tissue contains abnormally high concentrations of biotin (West and Woglom, 1941). However, growth of spontaneous mammary cancer in mice was not influenced by the induction of a biotin deficient state (Kensler *et al.*, 1943). The feeding of large amounts of egg white did not cause the regression of neoplastic tissue in patients nor was the excretion of biotin abnormally low (Rhoads and Abels, 1943).

Pteroylglutamic Acid. The toxicology and pharmacology of pteroylglutamic acid were investigated by Harned *et al.* (1946). Due to the

low order of solubility the compound was administered as its sodium salt. As with the other members of the vitamin B complex, pteroylglutamic acid possesses a low acute and chronic toxicity and an almost complete absence of side reactions. The acute intravenous LD 50 in mg./kg. for the several species is as follows: mouse, 600 mg./kg.; rat, 500 mg./kg.; rabbit, 410 mg./kg.; guinea pig, 120 mg./kg. Thus the mouse can tolerate 5 times as much of the compound as the guinea pig. Delayed deaths were encountered in both guinea pigs and rabbits. This observation, coupled with an inverse relationship between toxicity and water intake in all species studied, suggested renal damage as a contributing factor. Pathological examination revealed that a yellow substance, apparently pteroylglutamic acid, had been precipitated in the tubules. The deposition of this compound in the kidney is similar to that observed with another sparingly soluble vitamin namely, riboflavin.

Some animals, particularly rats, died within 30 minutes after injection. Death followed a violent convulsion which was predominantly tonic.

The chronic toxicity of pteroylglutamic acid was studied in rabbits and in rats. Rabbits receiving 5 mg./kg. daily by the peritoneal route for a period of 7 weeks remained normal in all respects. When rabbits were given 50 mg./kg./day for 10 weeks a questionable retardation in growth was observed. The blood picture and general appearance were the same as with the controls. The animals at autopsy, however, showed signs of renal injury probably due to obstruction.

Some depression in growth was noted in rats given 75 mg./kg. of pteroylglutamic acid daily by the intraperitoneal route. As was the case with the rabbits, renal damage was observed at autopsy. The dosage employed in this test was far above the clinically effective range. (The prescribed daily dose in humans is 5 mg.: a 200 g. rat was given 15 mg.)

Pteroylglutamic acid did not have a significant effect upon the blood pressure or respiration of dogs, cats, or rabbits. The compound had a very low order of activity on isolated strips of rabbit intestine. The intracutaneous injection of the sodium salt produced no irritation.

Pteroylglutamic acid has been employed widely in nutrition studies following its isolation by Pfiffner *et al.* (1943) and by Stokstad (1943). The most vital of its properties is that as a hematopoietic factor in the treatment of certain macrocytic anemias in man: sprue, anemia of pregnancy, and nutritional anemia. The compound is also effective in the therapy of the dyscrasia produced in rats by the feeding of purified rations containing sulfaguanidine or succinyl sulfathiazole (Daft and Sebrell, 1943). Anemias which are produced experimentally in the

chick (Piffner *et al.*, 1943; Stokstad, 1943) and in the monkey (Day *et al.*, 1945) respond to pteroylglutamic acid. Numerous papers on the above subjects have appeared since the synthesis of folic acid was announced by Angier *et al.* (1945).

Although in experimental animals the efficacy of pteroylglutamic acid and of structurally related compounds is ascribable to the correction of a deficiency state, its mode of action in the treatment of certain macrocytic anemias in man is unknown. On the basis of urinary excretion figures (Wright and Welch, 1943; Johnson *et al.*, 1945) the intake of this vitamin, under ordinary circumstances, would greatly exceed the need for it. In man, pteroylglutamic acid has been effectively employed in the treatment of the macrocytic anemias of pregnancy (Moore *et al.*, 1945) and sprue (Darby and Jones, 1945). In Addisonian (pernicious) anemia the blood dyscrasia responds to folic acid therapy, but the occurrence of the degenerative changes of the spinal cord and peripheral nerves remain unaffected by the administration of even massive doses of folic acid, although a prompt response is elicited following liver therapy (Vilter *et al.*, 1946; Heinle and Welch, 1947; Meyer, 1947).

An increase in the leukocyte count was noted after the administration of folic acid to patients who developed leukopenia following roentgen-ray therapy (Watson *et al.*, 1945).

Pteroylglutamic acid was reported to stimulate the growth of sarcoma 180 transplants in female Rockland mice while pteroyldiglutamyl glutamic acid caused a regression of the tumors (Lewisohn *et al.*, 1946). This work still lacks confirmation. Farber *et al.* (1947) have presented a preliminary report dealing with clinical trials of pteroyldiglutamic acid and pteroyltriglutamic acid in man. The findings relative to tumor growth are not convincing. The adult patients, however, gave evidence of subjective improvement following the administration of the compounds. Furthermore, both preparations appeared to reduce the requirement for analgesics.

Choline. The role of choline as a dietary essential is confused by the fact that mammals must be maintained on a ration low in protein in order to produce a deficiency state ascribable to a lack of choline. Methionine can assume the function of supplying a labile methyl group in the biosynthesis of choline.

Toxicity of choline chloride has been recently studied by Hodge and Goldstein (1942), Hodge (1944) and Neuman and Hodge (1945). The LD 50 is approximately 320 mg./kg. intraperitoneally for albino mice and 6.7 gm./kg. orally for rats. An increase of toxicity with concentration was found regardless of whether the choline was given orally or

intraperitoneally. Among the toxic manifestations were respiratory paralysis and the excretion of a protoporphyrin-containing discharge from the Harderian glands.

Chicks fed diets containing 1, 2, and 4% choline showed a progressive depression in growth. Although gross pathological lesions were not observed a decrease in body fat was noted which was more marked as the level of choline was increased (Melass *et al.*, 1946).

Davis (1944, 1946) claimed to have induced a hyperchromic anemia in dogs by the oral administration of choline. However, Clarkson and Best (1947) failed to confirm Davis' findings. Livingstone and Witts (1945) were unable to produce an anemia in rabbits with choline.

It is of interest to note that Moosnick *et al.* (1945) reported the successful treatment of a case of Addisonian pernicious anemia with choline chloride. Davis and Brown (1947) employed choline chloride in the therapy of three cases of (non-Addisonian) megaloblastic anemia. Although choline chloride in itself and parenteral administration of liver were without effect, the administration of liver extract if preceded by choline was followed by excellent blood regeneration.

2. Other Water Soluble Vitamins

Ascorbic Acid. It is generally accepted that ascorbic acid is virtually nontoxic. Intakes in excess of the bodily needs are excreted in the urine. Demole (1934) reported that guinea pigs tolerated doses of 0.4–2.5 g. ascorbic acid/kg. daily by the oral, subcutaneous, or intravenous routes, amounts representing 500–1000 times the guinea pig's requirement for ascorbic acid. Mice receiving 0.5–1.0 g./kg. for 7 days were indistinguishable from their controls throughout the experimental period and for a fortnight thereafter. Histological examination of the tissues revealed no abnormalities. Large quantities of ascorbic acid were likewise well tolerated by rabbits, cats, and dogs. Abt and Farmer (1938) administered repeated doses of 1 to 6 g. ascorbic acid orally and intravenously to adults without evidence of toxic action. These investigators are of the opinion that vagotonic symptoms in children observed by Schade (1935) and Widenbauer (1936) are ascribable to idiosyncrasy or sensitivity to the vitamin. Maurer *et al.* (1938) gave daily doses of 1 to 3 g. ascorbic acid to 100 patients suffering from insomnia without evidence of toxic symptoms other than a slight drowsiness that disappeared 24 hours after the vitamin was discontinued.

Ascorbic acid has been reported to have a diuretic action (Abbasy, 1937; Evans, 1938). Its activity is less than that of the mercurial and xanthine diuretics, and ammonium chloride. The use of ascorbic acid

as a diuretic may be indicated when slow and progressive dehydration is desired.

The role of ascorbic acid in the formation of intracellular substance seems well established. The fact that reticulum and collagen are not formed in the scorbutic animal led to the successful trial of ascorbic acid in accelerating wound healing (Lanman and Ingalls, 1937; Lund and Crandon, 1941; Farmer, 1944; Lund, 1939; Bartlett *et al.*, 1940; Hoxworth, 1944; Bourne, 1944). Ascorbic acid apparently is also essential for the healing of fractures. The excretion of ascorbic acid falls rapidly in animals with experimental multiple fractures. The degree of healing is dependent upon maintaining an optimal level of ascorbic acid in the diet (Lauber *et al.*, 1937). The requirement for fracture repair in the guinea pig is essentially the same as in wound healing (Bourne, 1942).

Except in frank cases of scurvy it is doubtful if ascorbic acid has any effect upon tooth structure.

The reducing property of ascorbic acid may be responsible for its efficacy in the treatment of methaemoglobinemia (Deeny *et al.*, 1943; Graybiel *et al.*, 1945; Barcroft *et al.*, 1945; Lapage, 1946; Sievers and Ryon, 1945), arsphenamine poisoning (Sulzberger and Oser, 1935; Cohen, 1938; McChesney *et al.*, 1944; McChesney, 1945, Farmer *et al.*, 1940), lead-poisoning (Holmes *et al.*, 1939), benzene poisoning etc. (Libowitzky and Seyffried, 1940). Space does not permit a detailed discussion of these uses; furthermore, the therapeutic effects of ascorbic acid in these conditions, if existent, are probably nonspecific and not due to its function as a vitamin.

The high content of ascorbic acid in the adrenals led investigators to attempt to discover a functional relation between adrenocortical secretion and the production of ascorbic acid. Szent-Györgyi (1930) stated that ascorbic acid completely inhibited the formation of a pigment in all systems in which an melanoid pigment is formed through the oxidation of a phenol. Association of an ascorbic acid low state with the pigmentation of Addison's disease was a logical conclusion. A number of investigators (Morawitz, 1934; Hoff, 1936 and Rothman, 1942) reported a decrease in the pigmentation found in this disorder, following the administration of ascorbic acid. The urinary excretion of ascorbic acid was low in patients affected with Addison's disease and increased following the daily administration of 300–500 mg. of ascorbic acid (Wilkinson and Ashford, 1936). It is difficult, however, to evaluate the significance of low ascorbic acid excretion in view of the poor state of health and general nutrition of individuals with this malady.

Dugal and Therien (1947) have reported that the amount of ascorbic acid in the tissues of rats kept at low temperatures (24 and 39°F.)

increased by 80% over that of rats maintained at room temperature. Under these conditions, rats apparently synthesize more ascorbic acid to aid their body adaptation to cold. Guinea pigs were found to be able to withstand lowered temperature depending upon the amount of ascorbic acid which they received. A relation between adaptation to cold and the ascorbic acid concentration in the tissues, particularly the adrenals, was observed.

Ascorbic acid therapy has been employed with conflicting results in the treatment of varying types of infections as well as in the therapy of allergic conditions such as asthma and ragweed hay fever (Ruskin, 1945; Newbold, 1944).

Vitamin P (Citrin). Rusznyak and Szent-Gyorgyi (1936) reported that a flavone glucoside (citrin) which they had isolated from Hungarian red peppers and from lemon juice prolonged the life of ascorbic acid deficient guinea pigs and reduced the severity of the hemorrhages observed in scurvy. Their preparation was also effective in the treatment of the increased capillary permeability of purpura. A number of investigators, the first of whom was Zilva (1937) were unable to confirm the Hungarian workers' findings and Szent-Györgyi himself later reported his failure to repeat his original observations (Szent-Györgyi, 1938). However, more recently Zacho (1939), Rusznyak and Benko (1941), Bacharach *et al.* (1942) and Bourne (1943) who have employed a more precise method for measuring capillary fragility have demonstrated that vitamin P has this property.

Few data are available on the toxicity and pharmacology of the glucosides hesperidin and eriodictyol, which in varying proportions constitute vitamin P, citrin, and rutin. Garino (1913) administered to dogs rutin, citrin, and the glucosides, hesperidin and naringin by mouth or parenterally. None was found to be toxic. Recent investigations (Wilson *et al.*, 1947b) have demonstrated that adult rats and guinea pigs tolerated 30–50 mg./kg. of rutin by the intraperitoneal or intravenous routes of administration. Rabbits receiving 100–200 mg./kg. intravenously remained free from untoward effects.

Chronic toxicity studies were carried out with rats which had been placed on a diet containing rutin in the amount of 0.25–1%. Animals receiving such rations for long periods remained normal both in growth and in reproductive performance.

Wilson and DeEds (1940) fed naringin and hesperidin to rats for 200 days at a level of 1% in the diet without evidence of cumulative injury.

The intravenous administration of citrin caused a slight fall in blood pressure which could be ascribed to vasodilation (Armentano, 1938).

Vitamin P has been employed therapeutically in the treatment of

conditions in which increased capillary fragility has been observed, such as the purpura of rheumatic fever (Rinehart and Johnson, 1944) and retinal hemorrhages associated with hypertension (Griffith and Lindauer, 1944). Assessment of the value of rutin medication in hypertension is difficult due to the variability of capillary fragility in untreated cases. Furthermore, increased capillary fragility is not a common complication of hypertension and methods for the measurement of capillary resistance are crude.

Wilson *et al.* (1947a) have reported that rutin protects guinea pigs from histamine shock. The action, however, is indirect, as evidenced by the lack of protection when the two drugs are administered simultaneously. It is possible that this protection is due to a retardation of epinephrine destruction resulting in a slightly increased level in the blood. A recent report by Raiman *et al.* (1947) indicates that rutin may prevent anaphylaxis.

III. FAT SOLUBLE VITAMINS

1. Vitamin A

Harris and Moore (1928) and Collazo and Rodriguez (1933) described the sequence of changes occurring following overdosage with Vitamin A. Although cod liver oil and cod liver oil concentrates were employed in their studies, later investigations with pure preparations have confirmed their findings (Vedder and Rosenberg, 1938; Wolbach and Bessey, 1942; Wolbach, 1946). The administration of excess vitamin A produced roughening of the skin, rarefaction of the bones, alopecia, and profuse internal hemorrhage. Toxic doses of vitamin A accelerated, in general, those growth sequences of bone retarded by the deficiency (Wolbach, 1946).

Light *et al.* (1944) found that the hypoprothrombinemia produced by excessive vitamin A could be controlled by the simultaneous daily administration of vitamin K (2-methyl-1,4-naphthoquinone). This finding has been confirmed by Walker *et al.* (1947).

Signs of vitamin A intoxication in man described by Spiesman (1941) and Rodahl and Moore (1943) are malaise, loss of weight and appetite, headache, and giddiness.

As man relies on plants to a great extent for his source of vitamin A, mention should be made of conditions arising from an excessive ingestion of the carotenoids. This subject has been reviewed by Josephs (1944). When the carotene rises in the blood beyond a certain level (which varies with the individual) the skin becomes yellow to orange in color. This condition, the severity of which may be influenced by a coexisting patho-

logical state, is known as carotenemia. The horny layers of the skin are colored by the carotene excreted by the sebaceous glands. In contrast with jaundice, the conjunctiva, urine, and feces are not discolored.

Vitamin A concentrates have been employed extensively in the treatment of night blindness and in the therapy of follicular hyperkeratosis. However, the success encountered in such cases is ascribable to the correction of a deficiency state.

2. Vitamin D

Two steroids having vitamin D activity are of clinical interest. Vitamin D₂ (Calciferol) is the antirachitic principle produced by the irradiation of ergosterol, the main sterol of yeast. Vitamin D₃, activated 7-dehydrocholesterol, constitutes the vitamin D present in animal fats. All forms of natural and synthetic vitamin D are toxic when given in excessive dosage. Contaminants (toxisterol and tachysterol) of crude preparations may also produce untoward reactions. Pfannenstiel, as early as 1928, reported that peroral administration of large quantities of irradiated ergosterol to growing rabbits resulted in severe cachexia followed by death. Toxic manifestations of vitamin D overdosage have been reviewed by Reed *et al.* (1939).

Morgan *et al.* (1937) reported that in rats calciferol was more toxic than was the "vitamin D" of fish liver oil. Excess vitamin A appeared to decrease, but not eliminate the harmful effects of an excessive dose of irradiated ergosterol. Morgan *et al.* (1940) later found that large doses of vitamin D₃ in rats produced damage more rapidly than did vitamin D₂; however, symptomatic recovery occurred sooner from vitamin D₃ than from D₂ poisoning. Harris *et al.* (1939) found that the vitamin D of irradiated ergosterol was definitely more toxic than the vitamin D of tuna liver oil.

Young dogs are apparently more susceptible to excess vitamin D (Hendricks *et al.*, 1947) than adult animals.

Morgan *et al.* (1947) reported that young dogs were unable to tolerate a single dose of 300,000 to 500,000 units/kg. of irradiated ergosterol. Of eight 4- to 5-week old dogs employed in these studies three were dead within 2 weeks and the remainder showed signs of a high order of toxicity. Becks (1942 and Becks *et al.* 1946a) has observed that excess vitamin D has a deleterious effect upon dental and paradental structures. Even a single dose of vitamin D₂ (450,000 units administered to month-old puppies) produced osteoporosis of the mandible and degeneration of the teeth. Young dogs given excessive doses of vitamin D₂ or D₃ for a 5-month period showed profound pathological changes in dental structure. The abnormalities persisted following a recovery period of similar

duration (Becks *et al.*, 1946b). Single doses of vitamin D preparations (100,000 to 200,000 units/kg.) were employed by Wolf (1943, 1944) in the treatment of infantile rickets. These findings cast doubt upon the wisdom of employing massive doses of vitamin D in the treatment or prophylaxis of infantile rickets.

The potential danger of overdosage with vitamin D cannot be overstressed; 150,000–200,000 units as “Ertron” have been employed by Reed *et al.* (1939) in the treatment of arthritis. As pointed out by these investigators, there is a definite risk from such intakes. In fact, at least one death from intoxication with Ertron has been reported (Kaufman *et al.*, 1947).

3. Vitamin E

Perhaps the most striking chemical property of the tocopherols is their antioxidant activity (Olcott and Mattill, 1936). The tocopherols not only protect the fats in which they occur from oxidation, but also enhance the activity of vitamin A and carotene. The degree of synergism of the several tocopherols does not parallel their antisterility activity (Joffe and Harris, 1943; Hickman *et al.*, 1944). The relative antidystrophic potency of the several tocopherols is not known.

The pharmacological properties of synthetic α -tocopherol and its acetate were investigated by Demole (1938–39) who found neither to be toxic. Mice given orally 50 g./kg. of body weight of α -tocopherol in one day showed a weight loss of 2 to 3 g. which lasted 3 to 4 days and was ascribable to anorexia. Rats tolerated oral doses of 5 g./kg. which is 500 times larger than the effective antisterility dose in vitamin E deficient rats. Frogs, cats, rabbits, and dogs tolerated 0.2 g./kg. orally, subcutaneously, intramuscularly, or intraperitoneally.

There was no local reaction in these species except a transient hyperemia following intracutaneous injection in the ear. Oral dosage of vitamin E for 10 successive days was tolerated in a total dosage of 0.5–1 g./kg. by normal rats and 0.1 g./kg. by dogs and cats; 2.5 g./kg. subcutaneously and intraperitoneally and 25 g./kg. orally were tolerated by mice. A total dose of 4 g./kg. given orally for 2 months was well tolerated by male and female rats, as were 0.21 g. over a month by dogs and 0.25 g. by cats.

α -Tocopherol has been used clinically in the treatment of habitual abortion and of certain neuromuscular disorders. As early as 1931, Vogt-Möller in Denmark reported that wheat germ oil was effective in preventing abortions in two women with previous histories of several prematurely terminated pregnancies, an observation which he later (1939) confirmed with a larger number of cases. Since abortion may be due to many factors, therapy with the tocopherols is difficult to evaluate.

An examination of the numerous reports on the use of vitamin E in the treatment of neuromuscular disorders of varying etiologies can only lead to the conclusion that neither vitamin E alone nor in combination with other vitamins is beneficial in amyotrophic lateral sclerosis, progressive muscular atrophy or progressive muscular dystrophy.

The possible value of vitamin E in the treatment of heart disease has received recent publicity. Vogelsang and Shute (1946) and Molotchick (1947) reported the efficacy of large doses of α -tocopherol acetate (200–600 mg.) in patients with congestive heart failure. According to these investigators the compound produced a pronounced diuresis. The effect on coronary pain was attributed to either a direct action on the coronary vessels or to an alteration of the metabolism of the heart muscle.

The editors of the *J. Am. Med. Assoc.* have stated (1946) that nothing in the known pharmacological properties of vitamin E would lead one to suspect a vasodilating action, a myotonic effect, or an ability to repair damaged heart muscle in human beings.

4. Vitamin K

There are two naturally occurring forms of vitamin K, namely K₁ and K₂. Another quinone, 2-methyl-1,4-naphthoquinone (Menadione), because of its ready availability and lower cost, has been used extensively and almost interchangeably with the naturally occurring vitamin K; however, it should be pointed out that under certain conditions these compounds differ considerably quantitatively as well as qualitatively. The belief that these compounds were interchangeable was based on the like order of activity found in chicks. Subsequent investigations with other species indicate, however, that this is not necessarily the case.

The acute oral and parenteral toxicity of vitamin K₁, phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone) and menadione (2-methyl-1,4-naphthoquinone) were investigated by Molitor and Robinson (1940). The oral L.D. 50 in mice was approximately 0.2 g./kg. for phthiocol and 0.5 g./kg. for menadione. No lethal effect could be produced by vitamin K₁ in doses up to 25 g./kg. The daily feeding for 30 days of 0.35 g./kg. of phthiocol and 0.5 g./kg. of menadione were toxic. A marked fall of the erythrocyte count and hemoglobin was observed in rats fed 0.1 g./kg. of phthiocol and 0.35 g./kg. of menadione while vitamin K₁ failed to produce such an effect.

Shimkin (1941) in a study of the acute and chronic toxicity in mice of six naphthoquinones with vitamin K activity, reported that in high dosage the compounds were respiratory depressants. Acute vascular congestion resulted in hemorrhagic extravasation in the renal tubules and in the liver.

Ansbacher *et al.* (1942) reported that the oral toxicities of menadione, menadiol, and its esters were approximately one-third to one-fifteenth of their subcutaneous toxicities. The manifestations of chronic damage were the result of injury to the red cells and not to the hematopoietic or other systems.

Schwarz and Ziegler (1944) observed that vitamin K lowers the blood pressure in hypertensive rats. Vitamin K in low concentrations has been reported to decrease the synthesis of acetylcholine (Torda and Wolff, 1945).

The various quinones with vitamin K activity have been employed therapeutically in the correction of hypoprothrombinemia, ascribable to a deficiency of vitamin K. While, as pointed out, menadione (2-methyl-1,4-naphthoquinone) is clinically most commonly employed and generally regarded as interchangeable with the true vitamin K, evidence is accumulating that vitamin K₁ or its oxide are more effective than menadione in counteracting the hypoprothrombinemia caused by administration of 2-(3-cyclohexyl-propyl)-3-hydroxy-1,4-naphthoquinone (Smith *et al.*, 1946) sulfaquinoxaline (Mushett and Seeler, 1947) or dicumarol (Mushett, personal communication).

Fosdick *et al.* (1942) reported that small amounts of 2-methyl-1,4-naphthoquinone prevented the production of acid when mixtures of saliva and glucose were incubated. Armstrong *et al.* (1943) found that many naphtho- and benzoquinones shared this antibacterial property. Armstrong and Knutson (1943) and Calandra *et al.* (1944) reported that the inhibitory action of vitamin K on acid formation in saliva was independent of its properties as a vitamin. 1,4-naphthoquinone had an antibacterial effect somewhat superior to 2-methyl-1,4-naphthoquinone, although the former compound does not possess more than 10% of the vitamin K potency of the latter.

The use of vitamin K preparations in the treatment of dental caries has resulted in considerable although unjustifiable publicity by the popular press.

REFERENCES

- Abbasy, M. A. 1937. *Biochem. J.* **31**, 339-342.
Abbasy, M. A., Harris, L. J., Ray, S. N., and Marrack, J. R. 1935. *Lancet*, **2**, **229**, 1399-1405.
Abderhalden, E., and Abderhalden, R. 1938. *Klin. Wochschr.* **17**, 1480.
Abt, A. F., and Farmer, C. J. 1938. *J. Am. Med. Assoc.* **111**, 1555-1565.
Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., Waller, C. W., Cosulich, D. B., Fahrenbach, M. J., Hultquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1945. *Science* **102**, 227-228.
Anigstein, L., and Bader, M. N. 1945. *Science* **101**, 591-592.

- Ansbacher, S., Corwin, W. C., and Thomas, B. G. H. 1942. *J. Pharmacol. Exptl. Therap.* **75**, 111-124.
- Antopol, W. 1942. *J. Med. Soc. New Jersey* **39**, 285-287.
- Antopol, W., and Unna, K. 1942. *Cancer Research* **2**, 694-696.
- Armentano, L. 1938. *Klin. Wochschr.* **17**, 1662-1663.
- Armstrong, W. D., and Knutson, J. W. 1943. *Proc. Soc. Exptl. Biol. Med.* **52**, 307-310.
- Armstrong, W. D., Spink, W. W., and Kahnke, J. 1943. *Proc. Soc. Exptl. Biol. Med.* **53**, 230-234.
- Atkinson, M. 1941. *J. Am. Med. Assoc.* **116**, 1753-1760.
- Axelrod, A. E., Carter, B. B., McCoy, R. H., and Geisinger, R. 1947. *Proc. Soc. Exptl. Biol. Med.* **66**, 137-140.
- Bacharach, A. L., Coates, M. E., and Middleton, T. R. 1942. *Biochem. J.* **36**, 407-412.
- Barcroft, H., Gibson, Q. H., Harrison, D. C., and McMurray, J. 1945. *Clin. Sci.* **5**, 145-157.
- Bartlett, M. K., Jones, C. M., and Ryan, A. E. 1940. *Ann. Surg.* **111**, 1-26.
- Becks, H. 1942. *J. Am. Dental Assoc.* **29**, 1947-1968.
- Becks, H., Collins, D. A., and Axelrod, H. E. 1946a. *Am. J. Orthodontics Oral Surg.* **32**, 452-462.
- Becks, H., Collins, D. A., and Freytag, R. M. 1946b. *Am. J. Orthodontics Oral Surg.* **32**, 463-471.
- Bicknell, F., and Prescott, F. 1946. *The Vitamins in Medicine*, 2nd Ed. William Heinemann, London.
- Bingham, A. W. 1936. *Am. J. Obstet. Gynecol.* **32**, 144-148.
- Black, S., McKibben, J. M., and Elvehjem, C. A. 1941. *Proc. Soc. Exptl. Biol. Med.* **47**, 308-310.
- Black, S., Overman, R. S., Elvehjem, C. A., and Link, K. P. 1942. *J. Biol. Chem.* **145**, 137-143.
- Bourne, G. H. 1942. *J. Physiol.* **101**, 327-336.
- Bourne, G. H. 1943. *Nature* **152**, 659-660.
- Bourne, G. H. 1944. *Lancet* **1**, 688-692.
- Bowman, K. M., Goodhart, R., and Jolliffe, N. 1939. *J. Nervous Mental Disease* **90**, 569-575.
- Brazda, F. G., and Coulson, R. A. 1946. *Proc. Soc. Exptl. Biol. Med.* **62**, 19-20.
- Briggs, G. M., Jr., Luckey, T. D., Mills, R. C., Elvehjem, C. A., and Hart, E. B. 1943. *Proc. Soc. Exptl. Biol. Med.* **52**, 7-10.
- Calandra, J. C., Fancher, O. E., and Fosdick, L. S. 1944. *J. Dental Research* **23**, 31-37.
- Calder, R. M. 1947. *Proc. Soc. Exptl. Biol. Med.* **65**, 76-83.
- Caldwell, F. E., and György, P. 1943. *Proc. Soc. Exptl. Biol. Med.* **53**, 116-119.
- Caldwell, F. E., and György, P. 1947. *J. Infectious Diseases* **81**, 197-208.
- Caldwell, W. A., and Hardwick, S. W. 1944. *J. Mental Sci.* **90**, 95-108.
- Calvert, L. S. 1946. *Brit. Med. J.* **1**, 422.
- Clarkson, M. F., and Best, C. H. 1947. *Science* **105**, 622-623.
- Cohen, M. B. 1938. *J. Allergy* **10**, 15-26.
- Collazo, J. A., and Rodriguez, J. S. 1933. *Klin. Wochschr.* **12**, 1732-1734; 1768-1771.
- Crittenden, P. J. 1948. *Arch. Intern. pharmacodynamie* **76**, 417-423.
- Daft, F. S., and Sebrell, W. H. 1943. *U.S. Pub. Health Service. Pub. Health Repts.* **58**, 1542-1545.

- Darby, W. J., and Jones, E. 1945. *Proc. Soc. Exptl. Biol. Med.* **60**, 259-260.
- Davidson, C. S., and MacDonald, H. 1943. *New Engl. J. Med.* **229**, 353-355.
- Davis, J. E. 1944. *Am. J. Physiol.* **142**, 402-406.
- Davis, J. E. 1946. *Am. J. Physiol.* **147**, 404-411.
- Davis, L. J., and Brown, A. 1947. *Blood* **2**, 407-425.
- Day, P. L., Mims, V., Totter, J. R., Stokstad, E. L. R., Hutchings, B. L., and Sloane, N. H. 1945. *J. Biol. Chem.* **157**, 423-424.
- Deeny, J., Murdock, E. T., and Rogan, J. J. 1943. *Brit. Med. J.* **1**, 721-723.
- Demole, V. 1934. *Biochem. J.* **28**, 770-773.
- Demole, V. 1938a. Kongressber. II, XVI. Intern. Physiol. Congresses, 19.
- Demole, V. 1938b. *Z. Vitaminsforsch.* **7**, 138-143.
- Demole, V. 1938-39. *Z. Vitaminsforsch.* **8**, 338-341.
- Diehl, F. 1937. *Münch. med. Wochschr.* **84**, 718.
- Dry, T. J. 1946. *Proc. Central Soc. Clin. Res.* **19**, 69-70.
- Dry, T. J., Butt, H. R., and Schreifley, C. H. 1946. *Proc. Staff Meetings Mayo Clinic* **21**, 497-504.
- Dugal, L. P., and Therien, M. 1947. *Can. J. Research* **25**, 111-136.
- Durand, J. I., Spickard, V. W., and Burgess, E. 1939. *J. Pediat.* **14**, 74-78.
- du Vigneaud, V., Spangler, J. M., Burk, D., Kensler, C. J., Sugiura, K., and Rhoads, C. P. 1942. *Science* **95**, 174-176.
- Editorial. 1944. *Brit. Med. J.* **2**, 476-477.
- Editorial. 1946. *J. Am. Med. Assoc.* **131**, 746.
- Eisenstadt, W. S. 1942. *Minnesola Med.* **25**, 861-863.
- Emerson, G. A., and Smith, D. G. 1945. *J. Pharmacol. Exptl. Therap.* **85**, 336-342.
- Evans, W. 1938. *Lancet* **1**, 308-309.
- Farber, S., Cutler, E. C., Hawkins, J. W., Harrison, J. H., Peirce, E. C., 2nd, and Lenz, G. G. 1947. *Science* **106**, 619-621.
- Farmer, C. J. 1944. *Federation Proc.* **3**, 179-188.
- Farmer, C. J., Abt, A. F., and Aron, H. C. S. 1940. *Proc. Soc. Exptl. Biol. Med.* **44**, 495-499.
- Faust, E. C. 1946. *J. Am. Med. Assoc.* **132**, 965-969.
- Ferree, J. W., Weissman, N., Parker, D., and Owen, P. S. 1942. *J. Clin. Invest.* **21**, 401-408.
- Field, H., Jr., and Robinson, W. D. 1940. *Am. J. Med. Sci.* **199**, 275-276.
- Fosdick, L. S., Fancher, O. E., and Calandra, J. C. 1942. *Science* **96**, 45.
- Frostig, J. P., and Spies, T. D. 1940. *Am. J. Med. Sci.* **199**, 268-274.
- Garino, M. 1913. *Z. physiol. Chem.* **88**, 1-8.
- Goldzieher, J. W., and Popkin, G. L. 1946. *J. Am. Med. Assoc.* **131**, 103-105.
- Graybiel, A., Lilienthal, J. L., Jr., and Riley, R. L. 1945. *Bull. Johns Hopkins Hosp.* **76**, 155-162.
- Greiff, D., Pinkerton, H., and Moragues, V. 1944. *J. Exptl. Med.* **80**, 561-574.
- Griffith, J. Q., Jr., and Lindauer, M. A. 1944. *Am. Heart J.* **28**, 758-762.
- Hagiesco, D., Bazavan, G., Criscota, M., and Cioranescu, M. 1938. *Presse méd.* **46**, 1435-1438.
- Haley, T. J., and Flesher, A. M. 1946. *Science* **104**, 567-568.
- Hamilton, H. L. 1945. *Proc. Soc. Exptl. Biol. Med.* **59**, 220-226.
- Hamilton, H. L., Plotz, H., and Smadel, J. E. 1945. *Proc. Soc. Exptl. Biol. Med.* **58**, 255-262.
- Harned, B. K., Cunningham, R. W., Smith, H. D., and Clark, M. C. 1946. *Ann. N. Y. Acad. Sci.* **48**, 289-298.
- Harris, L. J., and Moore, T. 1928. *Biochem. J.* **22**, 1461-1477.

- Harris, R. S., Ross, B. D., and Bunker, J. W. M. 1939. *Am. J. Digestive Diseases* **6**, 81-83.
- Hecht, G., and Weese, H. 1937. *Klin. Wochschr.* **16**, 414-415.
- Heinle, R. W., and Welch, A. D. 1947. *J. Am. Med. Assoc.* **133**, 739-741.
- Hendricks, J. B., Morgan, A. F., and Freytag, R. M. 1947. *Am. J. Physiol.* **149**, 319-332.
- Hendricks, W. J., and Peters, M. 1947. *J. Pediat.* **30**, 72-75.
- Herzberg, B. 1941. *Southwestern Med.* **25**, 50.
- Hickman, K. C. D., Kaley, M. W., and Harris, P. L. 1944. *J. Biol. Chem.* **152**, 303-311; 313-320; 321-328.
- Hodge, H. C. 1944. *Proc. Soc. Exptl. Biol. Med.* **57**, 26-28.
- Hodge, H. C., and Goldstein, M. R. 1942. *Proc. Soc. Exptl. Biol. Med.* **51**, 281-282.
- Hoff, F. 1936. *Deut. med. Wochschr.* **62**, 129-134.
- Holmes, H. N. 1944. *Ohio State Med. J.* **40**, 237-238.
- Holmes, H. N., and Alexander, W. 1942. *Science* **96**, 497-499.
- Holmes, H. N., Campbell, K., and Amberg, E. J. 1939. *Science* **89**, 322-323.
- Hoxworth, P. 1944. *J. Am. Med. Assoc.* **124**, 483-485.
- Hunt, R., and Renshaw, R. R. 1929. *J. Pharmacol. Exptl. Therap.* **35**, 75-98.
- Jackson, B., and Wald, G. 1942. *Am. J. Physiol.* **135**, 464-473.
- Joffe, M., and Harris, P. L. 1943. *J. Am. Chem. Soc.* **65**, 925-927.
- Johnson, B. C., Hamilton, T. S., and Mitchell, H. H. 1945. *J. Biol. Chem.* **159**, 425-429.
- Johnson, W. M. 1945. *J. Am. Med. Assoc.* **129**, 91.
- Jolliffe, N. 1940. *Trans. Am. Neurol. Assoc.* **66**, 54-59.
- Jolliffe, N. 1941. *J. Am. Med. Assoc.* **117**, 1496-1502.
- Jolliffe, N., Bowman, K. M., Rosenblum, L. A., and Fein, H. D. 1940. *J. Am. Med. Assoc.* **114**, 307-312.
- Jolliffe, N., Goodhart, R., Gennis, J., and Cline, J. K. 1939. *Am. J. Med. Sci.* **198**, 198-211.
- Jolliffe, N., Rosenblum, L. A., and Sawhill, J. 1942. *J. Investigative Dermatol.* **5**, 143-148.
- Josephs, H. W. 1944. *Am. J. Diseases Children* **67**, 33-43.
- Kalaja, L. 1941. *Acta Med. Scand.* **107**, 427-498.
- Karl, R. C., Peabody, G. E., and Wolff, H. G. 1945. *Science* **102**, 12-14.
- Kaufman, P., Beck, R. D., and Wiseman, R. D. 1947. *J. Am. Med. Assoc.* **134**, 688-690.
- Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P. 1941. *Science* **93**, 308-310.
- Kensler, C. J., Wadsworth, C., Sugiura, K., Rhoads, C. P., Dittmer, K., and du Vigneaud, V. 1943. *Cancer Research* **3**, 823-824.
- Kinosita, R. 1937. *Trans. Soc. Path. Japon.* **27**, 665-725.
- Knott, E. M. 1936. *J. Nutrition* **12**, 597-611.
- Krehl, W. A., Teply, L. J., and Elvehjem, C. A. 1945a. *Science* **101**, 489-490.
- Krehl, W. A., Teply, L. J., and Elvehjem, C. A. 1945b. *Proc. Soc. Exptl. Biol. Med.* **58**, 334-337.
- Kuhn, R., and Boulanger, P. 1936. *Z. physiol. Chem.* **241**, 233-238.
- Lancet*, Annotation 1947. 223-224.
- Lanman, T. H., and Ingalls, T. H. 1937. *Ann. Surg.* **105**, 616-625.
- Lapage, C. P. 1946. *Brit. Med. J.* **1**, 293.
- Lauber, H. J., Nafziger, H., and Bersin, T. 1937. *Klin. Wochschr.* **16**, 1313-1315.

- Leitner, Z. A. 1943. *Lancet* **2**, 474-475.
- Levy, M. D., and Arnold, W. T. 1946. *Texas State J. Med.* **42**, 314-316.
- Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., and Keresztesy, J. C. 1946. *Science* **104**, 436-437.
- Libowitzky, O., and Seyfried, H. 1940. *Wiener Klin. Wochschr.* **53**, 543-547.
- Light, R. F., Alscher, R. P., and Frey, C. N. 1944. *Science* **100**, 225-226.
- Livingstone, S. M., and Witts, L. J. 1945. *Brit. Med. J.* **2**, 664-665.
- Loman, J., Rinkel, M., and Myerson, A. 1941. *Am. J. Med. Sci.* **202**, 211-216.
- Lord, J. W., Jr., and Andrus, W. DeW. 1941. *Arch. Internal Med.* **68**, 199-210.
- Lowry, O. H., and Bessey, O. A. 1944. *J. Biol. Chem.* **155**, 71-77.
- Lowry, O. H., and Bessey, O. A. 1946. *J. Biol. Chem.* **163**, 633-639.
- Lowry, O. H., Lopez, J. A., and Bessey, O. A. 1945. *J. Biol. Chem.* **160**, 609-615.
- Lucia, S. P., and Aggeler, P. M. 1944. *Proc. Soc. Exptl. Biol. Med.* **56**, 36-37.
- Lund, C. C. 1939. *New Engl. J. Med.* **221**, 123-127.
- Lund, C. C., and Crandon, J. H. 1941. *J. Am. Med. Assoc.* **116**, 663-668.
- McChesney, E. W. 1945. *J. Pharmacol. Exptl. Therap.* **84**, 222-235.
- McChesney, E. W., Barlow, O. W., and Klinck, G. J., Jr. 1944. *J. Pharmacol. Exptl. Therap.* **80**, 81-92.
- McCrea, F. D. 1938. *Sci. Proc. Am. Soc. Pharmacol. Exptl. Therap.* **63**, 25.
- Maisel, F. E., and Somkin, E. 1942. *J. Allergy* **13**, 397-403.
- Maroney, J. W., Davis, H. C., and Scott, E. G. 1946. *Delaware State Med. J.* **18**, 104-106.
- Mason, H. L., and Williams, R. D. 1942. *J. Clin. Invest.* **21**, 247-255.
- Maurer, S., Wiles, H. O., Schoeffel, E. W., and Fisher, M. L. 1938. *Illinois Med. J.* **74**, 84-85.
- Maxfield, J. R., Jr., McIlwain, A. J., and Robertson, J. E. 1943. *Radiology* **41**, 383-388.
- Melass, V. H., Pearson, P. B., and Sherwood, R. M. 1946. *Proc. Soc. Exptl. Biol. Med.* **62**, 174-176.
- Meller, C. L. 1942. *Minnesota Med.* **25**, 22-24.
- Melton, G. 1943. *Brit. Med. J.* **1**, 600-601.
- Meyer, H. H. 1935. Wesen und Sinn der exp. Pharmakologie, in Heffters Handbuch der exp. Pharmakologie. Erg. Werk. Bd., Springer-Verlag, Berlin, 1-10.
- Meyer, L. M. 1947. *J. Hematol.* **2**, 50-62.
- Mills, C. A. 1941. *J. Am. Med. Assoc.* **116**, 2101.
- Mitrani, M. M. 1944. *J. Allergy* **15**, 150-153.
- Molitor, H. 1942. *Federation Proc.* **1**, 309-315.
- Molitor, H., and Kuna, S. (Personal communication.)
- Molitor, H., and Robinson, H. J. 1940. *Proc. Soc. Exptl. Biol. Med.* **43**, 125-128.
- Molitor, H., and Sampson, W. L. 1936. *E. Merck's Jahresbericht* 51-64.
- Molitor, H., and Sampson, W. L. 1937. *Am. J. Physiol.* **119**, 377.
- Molitor, H., and Seeler, A. O. Unpublished data.
- Molotchick, M. B. 1947. *Med. Record* **160**, 667-668.
- Moore, C. V., Bierbaum, O. S., Welch, A. D., and Wright, L. D. 1945. *J. Lab. Clin. Med.* **30**, 1056-1069.
- Moosnick, F. B., Schleicher, E. M., and Petersen, W. E. 1945. *J. Clin. Invest.* **24**, 278-282.
- Morawitz, P. 1934. *Klin. Wochschr.* **13**, 324-327.
- Morgan, A. F., Axelrod, H. E., and Groody, M. 1947. *Am. J. Physiol.* **149**, 333-339.
- Morgan, A. F., Kimmel, L., and Hawkins, N. C. 1937. *J. Biol. Chem.* **120**, 85-102.

- Morgan, A. F., Shimotori, N., and Hendricks, J. B. 1940. *J. Biol. Chem.* **134**, 761-779.
- Murray, E. S., Z̄arafonetis, C. J. D., and Snyder, J. C. 1945. *Proc. Soc. Exptl. Biol. Med.* **60**, 80-84.
- Mushett, C. W. Personal communication.
- Mushett, C. W., and Seeler, A. O. 1947. *J. Pharmacol. Exptl. Therap.* **91**, 84-91.
- Nakahara, W., Mori, K. and Fujiwara, T. 1939a. *Gann* **33**, 13-17.
- Nakahara, W., Fujiwara, T., and Mori, K. 1939b. *Gann* **33**, 57-65.
- Neuman, M. W., and Hodge, H. C. 1945. *Proc. Soc. Exptl. Biol. Med.* **58**, 87-88.
- Neuwahl, F. J. 1942. *Lancet* **2**, 419-421.
- Newbold, H. L. 1944. *J. Allergy* **15**, 385-391.
- Olcott, H. S., and Mattill, H. A. 1936. *J. Am. Chem. Soc.* **58**, 1627-1630.
- Oppenheim, A., and Lih, B. 1946. *Radiology* **47**, 381-385.
- Page, R. C., and Bercovitz, Z. 1942. *Am. J. Med. Sci.* **203**, 566-569.
- Perla, D. 1937. *Proc. Soc. Exptl. Biol. Med.* **37**, 169-172.
- Perla, D. 1939. *Science* **89**, 132-133.
- Perla, D., and Sandberg, M. 1939. *Proc. Soc. Exptl. Biol. Med.* **41**, 522-527.
- Pfannenstiel, W. 1928. *Münch. med. Wochschr.* **75**, 1113-1114.
- Pfiffner, J. J., Binkley, S. B., Bloom, E. S., Brown, R. A., Bird, O. D., Emmett, A. D., Hogan, A. G., and O'Dell, B. L. 1943. *Science* **97**, 404-405.
- Pick, E. P., and Unna, K. 1945. *J. Pharmacol. Exptl. Therap.* **83**, 59-70.
- Pollack, M. A., Taylor, A., Taylor, J., and Williams, R. J. 1942. *Cancer Research* **2**, 739-743.
- Porter, C. C., Clark, I., and Silber, R. H. 1947. *J. Biol. Chem.* **167**, 573-579.
- Poser, E. F. 1942. *Wisconsin Med. J.* **41**, 490.
- Positano, G., and Ruggieri, G. 1942. *Boll. soc. ital. biol. sper.* **17**, 466.
- Raiman, R. J., Later, E. R., and Necheles, H. 1947. *Science* **106**, 368.
- Ravenel, S. F. 1947a. *J. Am. Med. Assoc.* **133**, 989-994.
- Ravenel, S. F. 1947b. *Southern Med. J.* **40**, 801-813.
- Reed, C. I., Struck, H. C., and Steck, I. E. 1939. Vitamin D. Chemistry, Physiology, Pharmacology, Pathology, Experimental and Clinical Investigations. University of Chicago Press, Chicago.
- Reeves, R. J. 1946. *Southern Med. J.* **39**, 405-407.
- Rhoads, C. P., and Abels, J. C. 1943. *J. Am. Med. Assoc.* **121**, 1261-1263.
- Richards, R. K. 1942. *Federation Proc.* **1**, 71-72.
- Rinehart, J. F., and Johnson, H. 1944. *J. Clin. Invest.* **23**, 941.
- Roch, M., and Scielounoff, F. 1938. *Schweiz. med. Wochschr.* **68**, 1343-1345.
- Rodahl, K., and Moore, T. 1943. *Biochem. J.* **37**, 166-168.
- Rose, H. M., Duane, R. B., and Fischel, E. E. 1945. *J. Am. Med. Assoc.* **129**, 1160-1161.
- Rosenblum, H., and Frazer, L. E. 1947. *Proc. Soc. Exptl. Biol. Med.* **65**, 178-180.
- Rothman, S. 1942. *J. Investigative Dermatol.* **5**, 67-75.
- Rudesill, C. L., and Weigand, C. G. 1941. *J. Indiana Med. Assoc.* **34**, 355-360.
- Ruskin, S. L. 1945. *Am. J. Digestive Diseases* **12**, 281-313.
- Rusznayak, St., and Benko, A. 1941. *Science*, **94**, 25.
- Rusznayak, St., and Szent-Györgyi, A. 1936. *Nature* **136**, 27.
- Sandground, J. H. 1943. *Science* **97**, 73-74.
- Schade, H. A. 1935. *Klin. Wochschr.* **14**, 60-61.
- Schmidt, F. R. 1945. *Arch. Dermatol. Syphilol.* **52**, 344-346.
- Schmidt, J. L., and Landy, M. 1942. *Proc. Soc. Exptl. Biol. Med.* **41**, 82-83.

- Schwarz, H., and Ziegler, W. M. 1944. *Proc. Soc. Exptl. Biol. Med.* **55**, 160-162.
- Scott, C. C., and Robbins, E. B. 1942. *Proc. Soc. Exptl. Biol. Med.* **49**, 184-186.
- Sebrell, W. H. (1941) 1943. *Research Pubs. Assoc. Research Nervous Mental Disease* **22**, 113-121.
- Sebrell, W. H., and Butler, R. E. 1938. *J. Am. Med. Assoc.* **111**, 2286-2287.
- Seeler, A. O. 1945. *J. Nat. Malaria Soc.* **4**, 13-19.
- Seeler, A. O., Mushett, C. W., Graessle, O., and Silber, R. H. 1944a. *J. Pharmacol. Exptl. Therap.* **82**, 357-363.
- Seeler, A. O., Ott, W. H., and Gundel, M. E. 1944b. *Proc. Soc. Exptl. Biol. Med.* **55**, 107-109.
- Service, W. C. 1946. *Ann. Allergy* **4**, 397-398.
- Shimkin, M. B. 1941. *J. Pharmacol. Exptl. Therap.* **71**, 210-214.
- Sievers, R. F., and Ryon, J. B. 1945. *Arch. Internal Med.* **76**, 299-307.
- Silber, R. H., and Unna, K. 1942. *J. Biol. Chem.* **142**, 623-628.
- Smith, C. B. 1946. *Can. Med. Assoc. J.* **54**, 589-591.
- Smith, C. C., Fradkin, R., and Lackey, M. D. 1946. *Proc. Soc. Exptl. Biol. Med.* **61**, 398-403.
- Smith, P. K. 1946. *J. Am. Med. Assoc.* **131**, 1114-1117.
- Snyder, J. C., Maier, J., and Anderson, C. R. 1942. Report to the Div. of Med. Sci. National Research Council, Dec. 26.
- Snyder, J. C., Yeomans, A., Clement, D. H., Murray, E. S., Zarafonitis, C. J. D., and Tierney, N. A. 1947. *Ann. Internal Med.* **27**, 1-27.
- Snyder, J. C., and Zarafonitis, C. J. D. 1945. *Proc. Soc. Exptl. Biol. Med.* **60**, 115-117.
- Spies, T. D., Bean, W. B., and Stone, R. E. 1938. *J. Am. Med. Assoc.* **111**, 584-592.
- Spies, T. D., Hightower, D. P., and Hubbard, L. H. 1940. *J. Am. Med. Assoc.* **115**, 292-297.
- Spiesman, I. G. 1941. *Arch. Otolaryngol.* **34**, 787-791.
- Stein, W., and Morgenstern, M. 1944. *Ann. Internal Med.* **20**, 826-828.
- Steinberg, C. L. 1938. *Am. J. Digestive Diseases* **5**, 680-681.
- Stoerk, H. C., Eisen, H. N., and John, H. M. 1947. *J. Exptl. Med.* **85**, 365-371.
- Stokstad, E. L. R. 1943. *J. Biol. Chem.* **149**, 573-574.
- Sulzberger, M. B., and Oser, B. L. 1935. *Proc. Soc. Exptl. Biol. Med.* **32**, 716-719.
- Sure, B. 1940. *J. Nutrition* **19**, 57-69.
- Sydenstricker, V. P., Schmidt, H. L., Fulton, M. C., New, J. C., and Geeslin, L. 1938. *Southern Med. J.* **31**, 1155-1163.
- Szent-Györgyi, A. 1930. *Science* **72**, 125-126.
- Szent-Györgyi, A. 1938. *Z. physiol. Chem.* **255**, 126-131.
- Taylor, J., and Becker, E. R. 1948. *J. Infectious Diseases* **82**, 42-44.
- Tierney, N. A. 1946. *J. Am. Med. Assoc.* **131**, 280-285.
- Tierney, N. A. 1947. *Southern Med. J.* **40**, 81-83.
- Tisdall, F. F., McCreary, J. F., and Pearce, H. 1943. *Can. Med. Assoc. J.* **49**, 5-13.
- Torda, C., and Wolff, H. G. 1945. *Proc. Soc. Exptl. Biol. Med.* **58**, 163-165.
- Trager, W. 1943a. *Science* **97**, 206-207.
- Trager, W. 1943b. *J. Exptl. Med.* **77**, 557-581.
- Turner, R. H., Snively, J. R., Grossman, E. B., Buchanan, R. N., and Foster, S. O. 1944. *Ann. Internal Med.* **20**, 193-218.
- Uhlmann, R. 1944. *J. Missouri Med. Assoc.* **41**, 218-221.
- Unger, P. N., and Shapiro, S. 1948. *J. Clin. Invest.* **27**, 39-47.
- Unna, K. 1939. *J. Pharmacol. Exptl. Therap.* **65**, 95-103.

- Unna, K. 1940. *J. Pharmacol. Exptl. Therap.* **70**, 400-407.
- Unna, K., and Greslin, J. 1940. *Proc. Soc. Exptl. Biol. Med.* **45**, 311-312.
- Unna, K., and Greslin, J. 1941. *J. Pharmacol. Exptl. Therap.* **73**, 85-90.
- Unna, K., and Greslin, J. 1942. *J. Pharmacol. Exptl. Therap.* **76**, 75-80.
- Unna, K., and Pick, E. P. 1944. *J. Pharmacol. Exptl. Therap.* **81**, 294-300.
- Vedder, A., and Rosenberg, G. 1938. *J. Nutrition* **16**, 57-68.
- Vilter, C. F., Vilter, R. W., and Spies, T. D. 1946. *Proc. Central Soc. Clin. Research* **19**, 26-27.
- Vogelsang, A., and Shute, E. V. 1946. *Nature* **157**, 772.
- Vogt-Möller, P. 1931. *Lancet* **2**, 182-183.
- Vogt-Möller, P. 1939. A Symposium Held under the Auspices of the Food Group (Nutrition Panel), Soc. Chem. Ind. London 57-66.
- Vorhaus, M. G., Williams, R. R., and Waterman, R. E. 1935. *J. Am. Med. Assoc.* **105**, 1580-1584.
- Vrtiak, E. G., and Lang, R. S. 1936. *J. Am. Med. Assoc.* **106**, 1828.
- Walker, S. E., Eyllenburg, E., and Moore, T. 1947. *Biochem. J.* **41**, 575-579.
- Watson, C. J., Sebrell, W. H., McKelvey, J. L., and Daft, F. S. 1945. *Am. J. Med. Sci.* **210**, 463-470.
- Weiss, S., and Wilkins, R. W. 1937. *Ann. Internal Med.* **11**, 104-147.
- Wells, J. J., and Popp, W. C. 1947. *Proc. Staff Meetings Mayo Clinic* **22**, 482-486.
- West, P. M., and Woglom, W. H. 1941. *Science* **93**, 525-527.
- Whipple, D. V., and Church, C. F. 1935. *J. Biol. Chem.* **109**, 98-99.
- Whitmore, W. H. 1943. *Am. J. Roentgenol.* **49**, 83-98.
- Widenbauer, F. 1936. *Klin. Wochschr.* **15**, 1158-1159.
- Wilkinson, J. F., and Ashford, C. A. 1936. *Lancet* **2**, 967-970.
- Williams, H. L. 1946. *Proc. Staff Meetings Mayo Clinic* **21**, 58-64.
- Williams, R. D., Mason, H. L., Wilder, R. M., and Smith, B. F. 1940. *Arch. Internal Med.* **66**, 785-799.
- Williams, R. H., Egana, E., Robinson, P., Asper, S. P., and Dutoit, C. 1943. *Arch. Internal Med.* **72**, 353-371.
- Williams, R. R., and Spies, T. D. 1938. Vitamin B₁ and Its Use in Medicine. MacMillan, New York.
- Wilson, R. H., and DeEds, F. 1940. *Food Research* **5**, 89-92.
- Wilson, R. H., Mortarotti, T. G., and DeEds, F. 1947a. *J. Pharmacol. Exptl. Therap.* **90**, 120-127.
- Wilson, R. H., Mortarotti, T. G., and Duxtader, E. K. 1947b. *Proc. Soc. Exptl. Biol. Med.* **64**, 324-327.
- Wolbach, S. B. 1946. *Proc. Inst. Med. Chicago* **16**, 118-145.
- Wolbach, S. B., and Bessey, O. A. 1942. *Physiol. Rev.* **22**, 233-289.
- Wolf, I. J. 1943. *J. Pediat.* **22**, 396-417; 1944. *ibid.*, **24**, 167-175.
- Wright, L. D., and Welch, A. D. 1943. *Am. J. Med. Sci.* **206**, 128-129.
- Wyatt, B. L., Hicks, R. A., and Thompson, H. E. 1936. *Ann. Internal Med.* **10**, 534-536.
- Yeomans, A., Snyder, J. C., Murray, E. S., Zarafonitis, C. J. D., and Ecke, R. S. 1944. *J. Am. Med. Assoc.* **126**, 349-356.
- Youmans, J. B., Patton, E. W., and Sutton, W. R. 1941. *Trans. Assoc. Am. Physicians* **56**, 377-383.
- Zacho, C. E. 1939. *Acta Path. Microbiol. Scand.* **16**, 144-155.
- Zilva, S. S. 1937. *Biochem. J.* **31**, 915-919.

The Assessment of Human Nutriture

By H. M. SINCLAIR

Wellcome Laboratory of Human Nutrition, University of Oxford

CONTENTS

	<i>Page</i>
I. Introduction.....	102
1. Definition of Terms.....	102
2. Physical State, Health, and Nutriture.....	104
3. Properties of Nutriture.....	105
4. Degrees of Nutriture.....	106
5. Duration of Nutriture.....	107
6. Development of Malnutriture.....	110
7. Methods of Assessing Nutriture.....	112
8. The Concept of Normality.....	112
II. Dietary Assessment.....	114
III. Chemical Assessment.....	116
1. Introduction.....	116
2. Composition of Tissues.....	117
3. Composition of Blood.....	118
4. Composition of Urine.....	120
IV. Functional Assessment.....	121
1. Introduction.....	121
2. Visual Acuity.....	121
3. Rod Threshold and Scotometry of the Dark-Adapted Eye.....	122
4. Capillary Fragility.....	124
5. Nerve Accommodation and Chronaxie.....	125
6. Ergometry, Endurance, and Ischemic Pain.....	126
7. Dynamometry.....	128
8. Cardiovascular and Respiratory Function.....	128
9. Psychometric Tests and Coordination.....	129
V. Somatometric Assessment.....	129
VI. Clinical Assessment.....	131
1. Mortality and Vital Statistics.....	131
2. Methods of Examination.....	132
3. General Assessment by Inspection.....	134
4. Symptoms.....	137
5. Signs.....	138
Skin, Hair and Nails.....	138
Tongue.....	141
Gums.....	142
Teeth.....	143
Mucous Membranes and Muco-cutaneous Junctions.....	144

	<i>Page</i>
Eyes.....	144
Glands.....	147
Nervous System.....	147
Connective Tissue, Muscle, Bone & Cartilage.....	148
Respiratory System.....	149
Circulatory System.....	149
Gastrointestinal System.....	149
Genitourinary System.....	150
VII. Discussion—Summary.....	150
Table 1. Arbitrary Standards of Normality Used by the Oxford Nutrition Survey.....	154
Table 2. Methods for Use in Rapid Assessments of Nutriture in Populations.....	156
References.....	157

I. INTRODUCTION

1. *Definition of Terms*

Nutrition is a process or action, nutriture is a state or condition; the state results from the process. The flow of a river into a basin produces a lake: the flow is a process and the lake is a result of that process. The flow may be increased by rainfall or impeded by a dam or fouled by an effluent; the lake will be altered accordingly. Without a basin there would be no lake: without a cell there can be no nutriture. The nutrition and therefore the nutriture of different types of cells of the body differ; and the nutriture of the body as a whole, which contributes so much to the health of the body, is the integration of the nutritures of the individual cells which contribute in different degrees. Before examining the state it will be well to examine the process.

Nutrition includes the process of metabolism in cells and everything contributing to this; it is therefore partly the sum of the processes

$$X \xrightarrow[\text{+ catalysts}]{\text{+O}_2} Y \text{ in a cell.}$$

But it is more than this: it is not restricted to the processes providing energy but also includes those that build new protoplasm or that make new compounds connected with the growth or metabolism of cells. Nutrition may obviously be disturbed if there is an improper concentration in cells of *X* or of oxygen or of the catalysts or of *Y*, or it may be disturbed by poisons that interfere with the reactions. The improper concentration of *X* may be caused by failure to ingest, digest, or absorb the substances that are oxidized, or that give rise after ingestion to products that are oxidized, with liberation of energy: carbohydrate, fat, and protein. These substances may reasonably be termed aliments.

An aliment may be defined as a nonspecific chemical compound that is used by the body for the production of energy and may in addition be used for growth or repair. A nutrient is a specific chemical substance that cannot be synthesized by the body in sufficient amount and must therefore be provided in relatively small amount unless a closely related substance is available to the body, since it or a closely related substance is essential for the bodily processes of metabolism, growth or repair. Nutrients include compounds of certain elements (such as Fe, Mg, Zn), certain amino acids, certain fatty acids, provitamins, and vitamins. Water and oxygen are also required by the body but are neither aliments nor nutrients.

Those compounds, aliments and nutrients, are contained in food. We may define food as what is ingested by the organism and provides one or more aliments and nutrients. A foodstuff is a substance that may be treated to form food. Thus a raw potato, a foodstuff, contains the aliment starch (which is not normally oxidized in the body but gives rise to glucose which is oxidized) and (amongst others) the nutrients β -carotene, ascorbic acid, and manganese; it may be boiled to provide a food, or it may be combined with another human foodstuff—a part of a cow—to form the food called cottage pie. A meal is the sum of one or more foods ingested at one period, and a diet is the sum of the daily or weekly meals considered especially in relation to their quality and effects. A dietary is a prescribed or adopted course of diet.

Our symbiotic microorganisms in the gut give rise to certain problems of definition. If urea is converted by bacteria in the gut into protein or polypeptides that are adsorbed, with or without preliminary digestion, are we to regard urea as an aliment? Similar problems may arise with cellulose and with the synthesis of nutrients by microorganisms. In all cases the precursors must be regarded as aliments or nutrients, and the microorganisms as contributing to true symbiosis. We must regard the activities of these microorganisms as a normal part of our economy, just as is the activity of pepsin. But it will usually be convenient, without further qualification, to regard the difference between nutrients as the difference between, say, manganese, thiamine, and calciferol, rather than between pyridoxal and pyridoxamine.

The nutrition of the cell depends then upon an adequate concentration of aliments within the cell or even within certain of its compartments; and this involves adequate ingestion or production in the gut, digestion, absorption, and then, perhaps after other processes, transport in the blood, diffusion into extracellular fluid and passage into the cell. The cell's nutrition also depends upon an adequate supply within the cell of certain nutrients, water, and oxygen, and upon the removal of waste products.

3. Properties of Nutriture

We must consider three important properties of nutriture: quality, degree, and duration.

Quality includes specificity. This, however, is not absolute, but arises from the fact that deficiency of a particular nutrient tends to produce a particular pattern thereby permitting clinical diagnosis. The pattern, however, will depend upon all the other properties of nutriture. There is no reason to doubt that deficiencies of two separate nutrients can independently produce a single clinical sign: angular stomatitis can apparently arise from deficiency of iron or of riboflavin. And this need arouse no surprise when it is appreciated that such a sign can be caused by a break in a metabolic chain, and this break can occur even at the same link in the chain through deficiency of either of two nutrients. In this sense specificity is not absolute. Moreover, it is wrong to suppose that the lesions produced by combined deficiencies are the sum of the lesions of the individual deficiencies. In rats deficiency of potassium or of thiamine causes myocardial necrosis, but deficiency of these two nutrients together is accompanied by no pathological change in the heart (Follis, 1942). Deficiency of niacin in man causes a scarlet tongue and deficiency of riboflavin causes a magenta tongue, but the tongue of the combined deficiencies will not have the tint that arises by mixing these two colors; indeed, when a pellagrin is treated with niacin without other dietary change his tongue may turn from bright red to magenta and angular stomatitis may appear, both events indicating a concomitant deficiency of riboflavin.

Quality expresses these kinds or types of deficiencies. In the case last mentioned, the quality of the malnutriture would be twofold: deficiencies of niacin and of riboflavin. Quality takes account of nutriture respecting the various nutrients and aliments, singly and in combination. If there are n different aliments and nutrients, and the different possible combinations of them as sufficient or deficient produce different qualities of malnutriture, the number of such qualities is 2^n . For man, n is probably at least 50, and therefore in theory there could be at least 10^{15} qualities. But the quality of nutriture is not only altered by deficiencies or combinations of deficiencies: poisoning with trivalent arsenic causes malnutriture of particular cells. Therefore the number of possible qualities becomes almost infinite, and it is necessary and indeed customary to consider certain typical ones. The types chosen, however, are often not as specific as is stated. It frequently happens that inanition accompanies deficiency of a specific nutrient, and therefore to the specific signs of this deficiency may be inadvertently added the

signs of deficiency of aliments: diminished adipose and lymphoid tissue, atrophy of the genital organs, diminished osteogenesis, and others.

Though the distinction is artificial, it is sometimes convenient to speak of general nutriture (of the whole body or of certain cells of the body), or of nutriture regarding specific nutrients. When we know more of the interplay of aliments and nutrients, and therefore of the quality of nutriture, the distinction will be needed less. The quality of nutriture pertaining to aliments alone is reasonably called nourishment, under-nourishment being a particular type of malnutriture. The word nutriment will be used to include aliments, nutrients, water and oxygen.

4. Degrees of Nutriture

Obviously there can be degrees of nutriture of individual cells or groups of cells, or of the body as a whole. It is desirable to define these degrees, and an interesting attempt to do so has been made by Dann and Darby (1945). I had also attempted this as follows and prefer to retain my classification for reasons that will be stated:

- (i) *Excess Nutriture*. The state in which impaired function or defective structure results from an excessive supply to certain cells of the body of one or more aliments or nutrients.
- (ii) *Normal Nutriture*. The state in which function and structure are unimpaired by nutrition, and the reserves are adequate for the usual needs of the body.
- (iii) *Poor Nutriture*. The state in which function and structure are unimpaired by nutrition, but the reserves are inadequate for the usual needs of the body.
- (iv) *Latent Malnutriture*. The state in which function or structure is impaired by nutrition, but disease exists only *in posse* or in an undeveloped form.
- (v) *Clinical Malnutriture*. The state in which impaired function or defective structure, produced by nutrition, causes definite disease, even though this may not be capable of diagnosis by a physical examination.

Death occurs at either end of the scale, from surfeit or deficiency.

Excessive ingestion of carbohydrate causes obesity; of fat, ketosis; of fluorine, brittle teeth; of iron, rickets; and hypervitaminoses have been described of various vitamins, such as A, D and thiamine. The degree called "saturation," used by Dann and Darby, should be avoided in a classification; there is no evidence that saturation is desirable and there is definite evidence that in some cases it is undesirable, as for instance in the examples just mentioned of the results of excessive ingestion; in

some cases, for example with carbohydrate or fat or vitamin A, it is not clear what saturation means. Dann and Darby include under "potential deficiency disease" the state in which a biochemical test reveals a decreased "ability of the organism to withstand a stress without deviation from its usual physiological course." The amount of ascorbic acid in 100 ml. of the plasma of a person saturated with this nutrient is about 1.7 mg.; if estimated in a person and found to be only 1.0 mg. it might reasonably be supposed that the person had decreased ability to withstand the stress of a scorbutic diet, yet one would hesitate to say that the person had potential scurvy. It is less unsatisfactory to define reserves in terms of the usual needs of the body in its usual environment: those who habitually live in arctic climates or who are accustomed to undergo dietary privations at certain seasons should have larger reserves than are needed by others; normal nutriture implies reserves that are adequate for the customary or current needs but are not necessarily maximal.

Even though function is unimpaired, the reserves of nutriment may be insufficient for the usual needs of the body. This state may be detectable biochemically by analysis of body fluids or tissues; or it may be detectable by subjecting the body to an insult that is not greater than the stresses it encounters in its usual activities and finding that function is now impaired whereas with increased reserves it would not be. This degree merges into the next in which structure or function is impaired in the absence of an added insult, but the person probably has no definite symptoms, and no clinical signs may be detected without the use of special tests of function or structure. This state corresponds to the "Latent deficiency disease" of Dann and Darby.

When there is definite deficiency disease, with marked structural changes in the tissues, the last state is reached: clinical malnutriture. Even in severe cases of this state, it may be necessary to establish the diagnosis by special tests, such as biochemical estimations, or by therapeutic trial.

It will be appreciated that the boundaries between the five degrees are arbitrary, and one degree merges into the next.

5. Duration of Nutriture

The last property of nutriture, duration, is concerned with the time during which the state has existed.

Some people (e.g., Kruse, 1943) fall into the error of classifying duration in terms of acuteness and chronicity. These are not determined by the duration of a particular degree of nutriture, but by the first differential coefficient of degree with respect to time. An acute or chronic process is one whose degree is changing rapidly or slowly. Except in the

rare case of a process that is alternately and rapidly advancing and receding in degree, an acute process cannot in general be of long duration because, if advancing, it will soon lead to death. If the degree of nutriture is plotted against time (Fig. 1), the slope of a line will determine rate of change of degree and therefore acuteness or chronicity. The evolution of the process or resulting state can be defined by any continuous line provided two rules are observed: first, the process or state cannot regress with respect to time; and secondly, we shall not allow the state to pass from death to one of the other degrees (a point probably overlooked by Kruse when he states that changes, whether acute or chronic, are reversible provided therapy is sufficient in amount and duration).

If rate of change (di/dt) of degree (i) with respect to duration (t) be plotted (Fig. 2), the position on the graph will determine the acuteness or chronicity of the process at a particular time.

It appears from clinical observation and from the experimental work on pigeons of Swank (Swank, 1940; Swank and Bessey, 1941; Prados and Swank, 1942), that acute deficiency of thiamine causes an accumulation of pyruvate in the lower parts of the brain and there follow capillary dilatation and then hemorrhages such as are found in Wernicke's encephalopathy; whereas chronic deficiency causes peripheral sensorimotor neuritis because the metabolism of the neurones is deranged, and if sufficiently prolonged permanent loss of function may result. Here we have a simple example of how rate of change and duration of malnutrition lead to two different states of malnutriture.

The mention of chronic lesions, and in particular of irreversible lesions, raises a difficulty. It is obvious that it is possible to have optimal nutrition coexistent with definite malnutriture: after severe deficiency has developed, therapy may restore all the metabolic processes to normal but if the processes of repair are continuing the clinical signs, though reversible, may still exist and therefore malnutriture may still be apparent. What then of permanent paralysis caused by athiaminosis or of permanent rachitic deformities? Since it is wrong to regard these as necessarily due to an existing malnutrition and confusing to regard them as manifestations of present malnutriture, it is convenient to restrict the latter term to states that are reversible by appropriate nutrition.

Kruse (1942a, 1943) has developed "a concept of the deficiency states" which may briefly be considered here. He appears to generalize about all deficiency states from his views concerning macroscopic and microscopic lesions in deficiencies of four vitamins: vitamin A, niacin, riboflavin, and ascorbic acid; the fact that these views are not acceptable is immaterial in this context. Kruse ascribes certain properties to the pathologic process in the tissue in deficiency disease, namely, velocity,

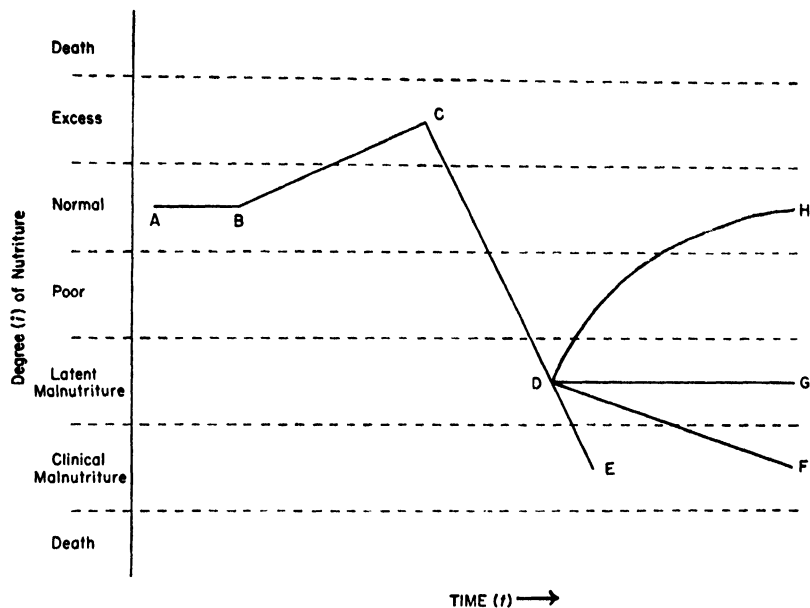


Fig. 1.

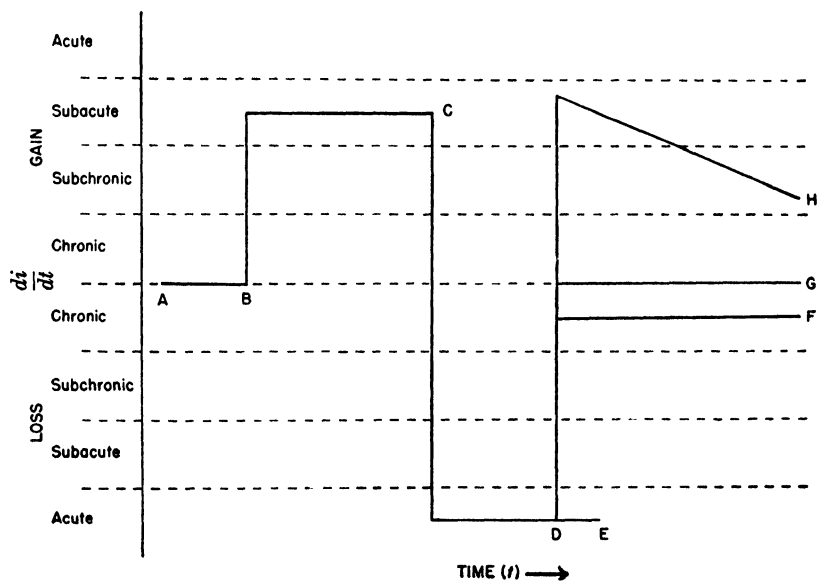


Fig. 2.

intensity, and duration. Velocity (also called "rate" and "form") gives rise to acute or chronic processes, "intensity" (also called "degree") to mild or severe processes, and "duration" is time. Velocity is determined by the causal force which is a function of degree and rate. It is at once apparent therefore that velocity cannot be synonymous with rate (or form) if it includes both rate and intensity. Further, since (as Kruse shows in a diagram) the essential difference between acuteness and chronicity is regarded as being in one dimension only, namely in time (or duration), the only dimension of velocity appears to be in time.

We must be clear in any such discussion whether we are considering the degree of deficiency of a nutrient in the diet or in the cells of the tissue, or the resulting degree of alteration in the process of nutrition in the cells, or the changed degree of nutriture produced by this alteration (i.e., the different state caused by the altered process). Each, of course, tends to follow the preceding one with inevitable temporal separation. With minor modifications the discussion in the earlier sections is applicable to the deficiency in the cells, to the process in the cells or to the resulting state. An acute deficiency in the cells, that is, a rapid change in concentration of nutriment in those cells, will produce a rapid change in the cellular metabolic processes and a state that may arise quickly and may be different in type from one that is caused by a slower alteration in the process. Conversely a slow change in concentration of nutriment in certain cells, which can be caused by an acute dietary deficiency when the relevant stores of the body are abundant, is likely to produce changes slowly in the metabolic processes with a nutriture of a different pattern.

Before further discussion it would be well to consider the general method of development of malnutriture.

6. Development of Malnutriture

It has been stated elsewhere (Sinclair, 1944) that malnutriture usually develops by stages which correspond to the above-mentioned degrees: first there is inadequate ingestion perhaps detectable by dietary methods and accompanied by decreased excretion, then decreased bodily reserves perhaps detectable by biochemical methods, then impaired function, then structural derangement that may be revealed at first by microscopical and later by gross anatomical changes.

These stages in development may be illustrated by an actual case, the later stages being added from experience with induced deficiency; their course may be followed on Figs. 1 and 2. Mary C., aged 24 years, was referred to me as a case of Eales' disease (angiopathia retinalis juvenalis) with the suggestion that it might be due to deficiency of ascorbic acid. Her history was that she had recently come from Ireland, and suddenly

developed misty vision in the right eye. On examination she was found to have haemorrhages into the posterior chamber of the right eye; she had marked xerosis of the skin of her arms of recent origin and her teeth had lately begun to break. Hypervitaminosis A was considered, but she admitted to taking no medicines. However, the dietist in the course of a diet history elicited the information that since coming from Ireland she had consumed daily over 60,000 I.U. of vitamin A in cod liver oil because she was told this would protect her against the English winter. She was in degree (i)—excess nutriture with impaired visual function, and impaired structure of capillaries, teeth, and skin, caused by hypervitaminosis A. In Ireland she had been travelling along the line AB, in England BC. Let us assume she stopped her self-medication (at point C) and, fearing vitamin A, excluded this and its precursors from her diet. She would pass through a transitory stage of normal nutriture, even though she retained some of the stigmata of the previous disease such as broken teeth; her plasma might contain 100 I.U. of vitamin A and 150 γ of carotenoids, and the rod threshold of her eye when fully dark-adapted might be $1.7 \log \mu\text{mL}$. Next the carotenoids in plasma would fall quickly to about 20; the plasma vitamin A might fall slightly and the rod threshold rise slightly but not significantly. She would now be in the degree of poor nutriture, detectable only by biochemical methods. The plasma carotenoids would remain approximately constant, the vitamin A would fall perhaps to 35 I.U., and the rod threshold would rise perhaps to $2.3 \log \mu\text{mL}$; this stage of partial night blindness and low plasma values indicates the fourth degree, latent malnutriture (point D): function is impaired but there is no definite deficiency disease. This, however, finally develops (point E). In the stage of clinical malnutriture the plasma vitamin A is very low, there is markedly deficient rod function, and clinical signs (such as xerosis conjunctivae, Bitot's spots, xeroderma, and perhaps follicular keratosis) may be present.

In this hypothetical example, deprivation is obviously acute. Let us assume that when Mary C. had reached point D (latent malnutriture) she slightly modified her diet to include some vitamin A, but less than her daily requirement; the deterioration would be slowed, becoming chronic (DF) rather than acute (DE). If she took just that amount of vitamin A and its precursors that equalled the daily utilization, the process might remain at a steady state with neither deterioration nor remission (DG); this is of course maximum chronicity since the rate of change is zero. If she improves her diet sufficiently, improvement in degree will occur (DH).

This process of improvement is not simple. The poor dark adaptation is in some cases cured in a matter of hours, a very acute response.

But if clinical changes had occurred, some might have responded slowly, some very slowly, and others not at all. As we have already said, if a particular lesion is reversible, the defect of nutriture cannot be said to have been completely remedied until it has disappeared, even though nutrition be normal.

7. Methods of Assessing Nutriture

We start from the premise that the full deficiency state, whether acute or chronic in origin, whether mild or severe in degree, usually develops in the four stages already mentioned: inadequate ingestion, decreased bodily reserves, impaired function, anatomical lesions. It follows that methods should be capable of detecting the abnormalities at these four stages: dietary, chemical, functional, and clinical methods (the word clinical being used in a restricted sense). In addition, we have somatometric methods, since before adulthood the growth of the body is profoundly affected by nutrition; and after adolescence changes, particularly in weight, occur as a result of nutrition. In subsequent sections it will be convenient to consider these five methods separately.

8. The Concept of Normality

Before considering the different methods of assessing nutriture it is necessary briefly to consider our standard for comparison. We have used the term "normal nutriture"; we have conceived of function being "impaired," of bodily needs being "usual," and of "definite disease" existing. Obviously the concept of disease and the diagnosis of disease imply that there is a state of nondisease, and this is customarily called the "normal" state of health.

There are three distinct concepts of normality, whether concerned with structure, function, or the chemical composition of the human body or its parts. The first is the concept of perfection: a "normal" person is one who is perfect according to some arbitrary set of standards. The view might merit careful consideration were it not that what is ideal or optimum in one set of circumstances may be a handicap in another. If deficiency of thiamine increases the resistance of the body to an attack of poliomyelitis, the ideal state when exposed to infection by the virus is quite different from the ideal state when partaking of a diet relatively high in carbohydrate. The ideal physical state for the marathon runner is obviously different from that of the German town dweller compelled to combat the rigors of another winter. Ideal ascorbic acid nutriture has been assumed by some to be the state of saturation in which the vitamin is spilled in the urine. The concept of perfection does not satisfy.

Secondly, according to the concept of pathology a person is "normal" who is free from the signs or symptoms of active disease; mild imperfections of the body are regarded as normal. According to Ryle (1947), the realm of the pathological includes structural or functional variants that are so pronounced as themselves "to occasion indisposition or inconvenience," or that have been "further increased or actually induced by disadvantageous stimuli or habit." Though perhaps satisfactory for the clinician, who as this word implies tends to be concerned with gross departures from normality found in patients in bed, the concept of pathology is unsatisfactory for the physiologist, as Ivy (1944) has shown in his very interesting discussion of normality.

Thirdly, the statistical concept of normality varies, as Ivy points out, depending on where it arbitrarily draws a dividing line between normal and abnormal. If a variate in a homogeneous population is measured, and if the variations are described by a Gaussian distribution curve with standard deviation σ , then about 68% of the population will fall within $\pm\sigma$ of the mean and about 95% within $\pm 2\sigma$. One or other of these limits is usually taken as arbitrarily describing the limits of normality. This sharp arbitrary distinction between normal and abnormal can be avoided by merely stating in statistical terms the extent to which any individual in the population deviates from the mean of the population in respect of the character measured. Though for many purposes the statistical concept is essential, it does not satisfy the customary meaning of normal for the physiologist: it would class dental caries as being normal in an adult population, and would probably class acne vulgaris as being normal in an adolescent population.

Ivy (1944) therefore states that "the body is in a physiologically abnormal state when its relatively steady state is disturbed." It would appear however that by this definition changes at puberty and the climacteric, as well as sleep or waking, would have to be regarded as abnormal physiologically. Ryle (1947) appears at times to adopt the concept of pathology (see above), at times the arbitrary statistical concept ("When . . . the thyroid gland is invisible in upwards of 93% of cases, it seems reasonable to regard invisibility as 'normal'"); although he writes of "the normal range" without defining it, he concludes that life "admits no constant and no norm." The physiologist, following Bernard, recognizes constants: the possession, for instance, by members of a human population of two eyes, biconcave erythrocytes, and (after infancy) an upright walk. If there is a "normal range," there must be a "norm."

I propose, following Ivy (1944), to adopt both a physiological and a statistical definition, in the latter case signifying "statistical normal" and

implying by this the limits not outside $\pm\sigma$ of the mean of a variate in a population of stated characteristics. Unless thus specified, normality will be defined in physiological terms: the condition of the body, or of some part or function of the body, may be said to be normal when it allows the usual functions to be adequately performed in the usual environment. The terms "usual" and "adequately" imply, of course, arbitrariness in the definition; "usual" means customary or current. Sometimes it will be convenient to use the statistical definition; the possession by an adult man of six digits on each hand or 50,000 leucocytes/ μ l. of blood might allow the usual functions to be adequately performed in the usual environment, but would be statistically abnormal.

II. DIETARY ASSESSMENT

Except in rare instances, dietary methods cannot alone be used to assess nutriture: in general their limitations are too great. Obviously, however, there are levels of intake below which malnutriture must occur in anyone: whoever eats no food for a month will undoubtedly suffer from deficiency of aliments. Dietary methods will therefore be mentioned only briefly.

Food consumption is estimated at various levels of distribution. Sometimes the "food moving into civilian consumption" is estimated by adding imported food and home-produced food, subtracting exported food and food used by noncivilians, and dividing the result by the civilian population. This crude method need not concern us. Food consumption may be estimated as distributed wholesale; or as purchased retail by the housewife; or as eaten after allowing for edible waste (the net result of losses and gains in cooking and of serving-waste) and inedible waste (which is preparation- and plate-waste), or as assimilated after allowing for further losses through excretion in urine and feces, and additions through aliments and nutrients synthesized or made available by bacteria in the gut. Obviously one must be clear at what stage food consumption is being assessed: there is confusion on this point even in Orr's classical study, "Food, Health and Income" (1936). Waste of various types must often be estimated, usually with considerable margins of error. For instance, waste that is allowed in most tables calculated on an "as purchased" basis is far too generous by wartime experience in England and especially by present experience on the continent of Europe.

There are five methods that are used to compute food consumption:

1. *National studies* in which the food resources of a country are computed and expressed *per capita* or by the use of some scale of man-equivalents.

2. *Institutional studies* in which the total food consumed by a homogeneous social group is computed.
3. *Budgetary studies* or family living studies, in which the amount of money actually spent, or calculated to be available for spending, on food is assessed and compared with the supposed cost of the cheapest adequate diet.
4. *Family studies* in which, usually, inventories of household stores at the beginning and end of a week are made, and food entering the house during this period is recorded; from this information, by various devices, the food consumed by the family is assessed.
5. *Individual studies* whereby the food consumed by a single person is assessed by one of three methods: first, by a detailed dietary history; secondly, by a questionnaire; or thirdly by weighing or otherwise measuring the meals consumed. This last method, which is the most accurate, has been developed in particular by Widdowson (Widdowson, 1936; Widdowson and McCance, 1936; 1942; McCance *et al.*, 1938; Widdowson and Alington, 1941; Widdowson, 1947).

The errors and advantages of these different methods cannot be discussed here.

But even if the precise amounts of food consumed were known, the problem of translating these into nutrients arises. The errors, which have been investigated by McHenry (Patterson and McHenry, 1941; Young and McHenry, 1942, 1943; Semmons and McHenry, 1944) and by Widdowson and McCance (1943), may be very large for sundry reasons which include variations in nutrient content of different types of a food-stuff (such as different sorts of apple or of jam) and incorrect allowances for losses in cooking; in the North Carolina survey, no allowance was made for ascorbic acid present in cooked food and therefore "an average daily intake of 23 mg. vitamin C, which is about one-half the minimum normal standard" was encountered (Milam, 1942); when the presence of ascorbic acid in cooked food was appreciated, "the resultant mean intake would seem to approach the recommended dietary allowance" (Milam and Darby, 1945).

The main interest in the assessment of the dietary intake of aliments and nutrients lies in estimating from the assessed intake the amount of these available in the body and comparing the available aliments and nutrients with the individual's supposed requirement for normal nutriture. The difficulties in assessing availability and requirements have been discussed by many authors (e.g., Pett, 1945), and some have been briefly mentioned elsewhere (Sinclair, 1947b) with particular reference

to calories. Perhaps dietary niacin may be cited as an example here. The National Research Council (1945) recommends 15 mg. daily of niacin as a dietary allowance for a moderately active man, this figure being based on the belief that the requirement of niacin is probably about ten times that of thiamine (for the requirement of which our knowledge is far from precise). There is evidence that niacin may be synthesized by bacteria in the gut and then absorbed (Benesch, 1945), that tryptophan is a precursor of niacin (Perlzweig *et al.*, 1947), and that a toxic factor may be involved in the production of pellagra (Woolley, 1946; Kodicek *et al.*, 1946). It is therefore impossible to compute the dietary requirement of niacin, and the dietary allowance will be a matter more of fancy than of fact. Thiamine is not only synthesized in the gut but also gets destroyed there: about half the thiamine in a meal may be destroyed if, for instance, raw clams are consumed at the same time (Melnick *et al.*, 1945). It therefore becomes extremely difficult to state how much of certain nutrients actually becomes available in the body, and what is the requirement for normal nutriture either in the food ingested or in the food absorbed from the gut. Also, physiological adaptation can occur and alter the requirement of nutrients (Mitchell, 1944). Finally, the biological action of certain aliments or nutrients in certain combinations is known to be different from that obtaining in other combinations; and the efficiency of utilization of ingested nutrients or aliments may differ under different circumstances, as shown, for instance, by Sure and Dichek (1941).

Although dietary methods cannot usually be used alone to assess nutriture, a dietary history is an essential part of such an assessment and must be included even in rapid nutritional surveys. To such surveys family dietary studies are a valuable addition, although it is not usually practicable to conduct them on more than a small sample of the subjects included. The purpose of including them is not to assess nutriture from their results, but to aid in interpreting the cause of and suggesting a remedy for any malnutriture found. Weighing or estimating by homely measures the food consumed by individuals is seldom practicable in such rapid surveys.

III. CHEMICAL ASSESSMENT

1. Introduction

In the chemical assessment of nutriture, substances may be estimated in tissues, blood, urine, or other fluids (such as milk, c.s.f., or sweat). The substances usually estimated fall into three groups: first, nutrients or aliments in the resting condition or after a test dose; secondly, normal

chemical constituents that are affected by nutrients or aliments; thirdly, metabolic products whether estimated in the fasting condition or after some treatment. Measurements of erythrocrit or leucocrit, or of the number or size of erythrocytes, or of the number and type of leucocytes, are not chemical but it is convenient to include them here.

The aliments and nutrients that are estimated include protein (or different classes of proteins), amino acids such as cystine, glucose, vitamin A, carotenoids, ascorbic acid, thiamine, cocarboxylase, niacin, riboflavin, vitamin E, calcium, and phosphorus. Compounds affected by nutrients include hemoglobin, alkaline phosphatase, and prothrombase; metabolic products include pyruvate. With some compounds, such as hemoglobin or pyruvate, variations can be caused by more than one nutrient. The commoner methods of estimation we use have been summarized elsewhere (Sinclair, 1942, 1947a); other accounts include Youmans and Patton (1942), Mickelsen (1943), and papers by R. F. Johnson and Bessey. The very important alterations in blood composition caused by certain anticoagulants, by stasis during venepuncture, and by storing the blood cannot be discussed here; nor can the effect of alterations in blood volume as caused for instance by posture, activity, or dehydration.

2. Composition of Tissues

The obvious but important general principle may be stated that the composition of tissues alters before structural changes occur. It is surprising therefore that so little attention has been paid to the analysis of tissues. Muscle or liver biopsies are not easy. Hair and nails (in men or in adequately shod women) are readily collected and might prove quite useful for certain purposes: in our surveys during the famine in the Netherlands we collected samples of hair to try to obtain information about the previous protein nutriture when the hair was being formed.

The easiest and most satisfactory living tissue to obtain in nutritional surveys is leucocytes. They may be collected by the method of Butler and Cushman (1940) and then weighed, or spun to the graduated tip of a centrifuge tube after laking the erythrocytes; in Bessey's microtechnique the quantity of leucocytes present in solution is judged by the phosphorus content (Bessey *et al.*, 1947), but the soundness of this technique has not been established. The leucocytes are particularly valuable for the water-soluble vitamins. In the case of ascorbic acid, the excellent study by Crandon showed (Crandon *et al.*, 1940) that on a diet that produced scurvy the plasma ascorbic acid reached zero after 41 days, and the leucocyte ascorbic acid after twice this time and only shortly before clinical signs developed; the ascorbic acid in whole blood fell more rapidly than the leucocyte values but less rapidly than the plasma ascorbic acid.

Vitamins of the B complex are also conveniently and profitably estimated in leucocytes, and the advantages of this must now be discussed.

3. Composition of Blood

Under this heading we may include estimations upon whole blood or upon plasma or serum. The leucocytes, which may be regarded as "living" cells whereas the erythrocytes may be regarded as "dead," normally constitute such a small proportion of whole blood that their contribution to whole blood analyses is slight. Even when the plasma value is low and the leucocyte value high, the leucocytes may contain only a quarter of the total ascorbic acid in blood although they may contain 150 times the amount in an equal weight of plasma; in a person saturated with ascorbic acid, the plasma may contribute three-quarters, the erythrocytes a fifth, and the leucocytes only a twentieth of the ascorbic acid in whole blood.

Compounds in plasma behave in two strikingly different ways. Some, such as carotenoids or ascorbic acid, tend to reflect the immediate past intake: on a diet low in the particular nutrient, the plasma concentration drops rapidly; and when the nutrient is added to the diet there is a quick rise in plasma. Others, such as niacin and riboflavin, and usually protein, remain at concentrations that are within the average or statistically normal range even though clinical malnutrition, such as pellagra or ariboflavinosis, may be present. That the values are within the statistically normal range does not mean that no fall has occurred when there has been diminished intake of the nutrient. We believe on the present scant evidence that when a person subsists on a diet low in protein the serum protein concentration may fall slightly and then remain within the statistically normal range, protein meanwhile being taken from the tissues; later, depletion of the tissues may lead to a fairly rapid fall. On the other hand it is probable that a diet deficient in particular amino acids, such as lysine, may cause a rapid fall in serum protein without the initial plateau. A diet very low in animal protein is certainly compatible with a serum protein value within the statistically normal range; this was shown in one case by Abelin and Rhyn (1942), and the Oxford Nutrition Survey observed it repeatedly in the Netherlands and Germany (Sinclair, 1948a). But we have sometimes found unusually high protein values in groups of malnourished persons, such as Dutch children evacuated to England during the famine in the Netherlands or returned war prisoners; the cause of these high values, which is not dehydration, is being investigated. A single estimation of serum protein in an individual, therefore, gives little information if it falls within the statistically normal range; a value of, say, 6.3 g./100 ml. may be the level found in a particular person

however rich his diet be in protein; or it may be the level found in a person who has subsisted on a grossly deficient diet and who previously had a higher level. On the other hand significant differences between the means of population groups that are fairly homogeneous, and similar except for diet, are important; and a slight fall in a longitudinal study on an individual is valuable provided the nondietary factors that affect protein values (which have been carefully investigated by Lange (1946)) are remembered.

A similar example with thiamine may be given from a joint experiment in which 11 volunteers were placed upon a diet very low in thiamine, an equal control group receiving a daily supplement of the vitamin. After 86 days, the control subjects had a mean value of 6.50 γ /100 ml. of blood, with a standard deviation of 0.87, while the levels in the experimental subjects had a mean value of 3.95, with a standard deviation of 0.61. The ranges of values were then 5.5 to 8.0 in the control group and 3.0 to 5.0 in the experimental group, and were therefore just distinct. The subject whose value was 5.0 had at the start a value of 7.5 and after therapy at the conclusion of the experiment 7.0: had he been found at random in the population one could not have inferred from a single blood analysis that he was on a diet deficient in thiamine, but a longitudinal study of the values for thiamine in blood allowed the 22 subjects to be sorted with complete correctness into the control and experimental groups at a time when an attempt to do so on the basis of longitudinal clinical studies placed the subjects at random in the two groups. Similar results were obtained with independent estimations of cocarboxylase in blood.

We need much more information about blood levels, for example why a certain individual has a serum protein concentration of 6.5 g./100 ml. whereas another may have one of 8.5, both being on excellent diets. Somatotype is probably one important factor, but there are many others. We do not know why hyperthermia causes a rapid depletion of vitamin A in the liver with a sudden fall in serum vitamin A, whereas a diet almost free of vitamin A and carotenoids may cause no fall in serum vitamin A for months, and yet on other occasions may cause a fall in days (see below under Section IV, 3). Age and sex certainly affect levels of hemoglobin and of alkaline phosphatase, and probably affect others.

The following tentative summary of the use of analyses in cross-sectional studies of nutriture in individuals may be given. Estimations of ascorbic acid and of carotenoids in serum behave similarly in that both reflect the immediate past intake; a zero value of either is compatible with full health (in the case of carotenoids, a zero value of those that are biologically active corresponds to about 12 γ as usually estimated per

100 ml. of serum); and the information provided by the estimations may be very helpful for dietary purposes but is not of much value for assessing nutriture. Serum vitamin A falls early in deficiency and therefore estimations are valuable although individual variations are large. Blood pyruvate, blood hemoglobin, and serum phosphatase (in children) alter fairly early in deficiency; more work is required upon serum alkaline phosphatase in children and in adults before much can be said about this somewhat mysterious "enzyme." There is probably a slight drop in total protein and in albumin fairly early when the diet is low in protein, but in cross-sectional studies this does not help to assess the nutriture of an individual person; and some undernourished persons have unusually high values. A fall in serum calcium is a late occurrence. In living tissues such as leucocytes the amount of ascorbic acid reaches low values only shortly before malnutrition occurs, and the same is probably true for thiamine, niacin, and riboflavin. Blood values for these three members of the vitamin B complex become low only very late in malnutrition and the same seems usually to be true of serum values for protein or albumin. It must be emphasized once more that, even when the obvious factors such as changes in blood volume are controlled, we remain profoundly ignorant of the nondietary causes of variations between individuals at the same time or in single individuals at different times.

4. Composition of Urine

Though we are ignorant of the interpretation of blood values in man, we are even more ignorant of the interpretation of urinary values. Estimations are done sometimes upon random samples of urine, sometimes on fasting specimens, sometimes on 24-hour specimens, and sometimes after one or more test doses. The methods used have been briefly summarized elsewhere (Sinclair, 1947a). The urinary excretion of water-soluble vitamins will in general reflect the immediate past intake, but this has no clear relation to the assessment of nutriture. There seems to be no reason why a person in full health should be expected to excrete any ascorbic acid; he is not expected to excrete glucose in the urine. We know little about the factors involved in load tests (in which the excretion after a test dose is followed) or saturation tests (in which the number of days needed to "saturate" the body is determined); until we know more about the metabolism of nutrients and the renal threshold for them, the results of load or saturation tests must be interpreted with caution; a novice indulging in a glucose saturation or load test might reach very remarkable conclusions. Johnson, who has had a large experience of urinary excretion tests, prefers estimations upon fasting urine to load tests in field surveys (Johnson *et al.*, 1945). Miller and Beach (1945)

concluded that the fasting excretion of ascorbic acid "fails as a measure of vitamin C status of human subjects"; for thiamine they preferred load tests to fasting estimations because "apparently there are complex factors influencing the fasting excretion which tend to vitiate the fasting excretion value as a reliable measure of the state of thiamine saturation of the subject"; they reached similar conclusions regarding riboflavin. Mickelsen *et al.* (1947) could not distinguish between intakes of 1 mg. or 2 mg. of thiamine by urinary analysis. On the other hand, Cayer *et al.* (1945) concluded that urinary excretion tests for B complex vitamins "assist materially in evaluating the nutritional status of any given individual"; and Kajdi *et al.* (1939) obtained good results with a load test for ascorbic acid. The fact that only part of ingested niacinamide is excreted as *N*¹-methylniacinamide is a serious disadvantage in the estimation of this substance as a measure of the adequacy of niacin nutriture.

It has been customary, for reasons of convenience, to administer a single test dose in a load test, usually by mouth. Because many subjects in England during the war excreted no extra ascorbic acid after a test-dose of the usual size, we used large amounts administered orally (33 mg./kg. body-weight). Lowry and his colleagues (Lowry *et al.*, 1946), in an excellent study relating the concentration of ascorbic acid in different parts of blood and in urine to the dietary level, correctly point out that it is desirable to administer large amounts orally, but given in many divided doses throughout the day; this technique is often not practicable.

IV. FUNCTIONAL ASSESSMENT

1. Introduction

Physiology studies the functions of the body, and anatomy its structure. In this section we are not concerned with alterations in structure unless they affect function, but the two cannot be completely separated. Furthermore, the section on Clinical Assessment obviously must include consideration of function of which the testing (as in eliciting a knee jerk or counting the pulse) is a very common part of a clinician's examination. Arbitrarily we shall consider capillary fragility in this section, and radiological investigations in the section on Clinical Assessment.

2. Visual Acuity

It has been abundantly proved that rapid diminution can occur as a result of malnutrition. From the extensive Japanese literature, there is no doubt that retrobulbar neuritis occurs in association with beriberi (Kagawa, 1938), but the relationship to deficiency of thiamine is obscure. Diminished visual acuity has been alleged to be caused by deficiency of

riboflavin (Kruse *et al.*, 1940), and has often been described in pellagra together with retrobulbar neuritis and optic atrophy with central scotomata (e.g., Calhoun, 1918) although there is no clear evidence that deficiency of niacin causes the changes. Diminished vision was noted in warm climates by Strachan (1888), Scott (1918) and many subsequent authors as a symptom accompanying nutritional neuropathy. Such loss of vision caused by retrobulbar neuritis was described in Sierra Leone by Wright (1928), in Nigeria in 1930 by Moore (1939, 1940), in British Guiana in 1932 by Browne (1939), in Ceylon in 1933 by Nicholls (1935), in Malaya in 1935 by Landor and Pallister (1935; Pallister, 1940), in Trinidad by Métivier (1941). Grande and Peraita (1941) recorded visual disturbances in nearly half of a series of cases of nutritional neuropathy in Madrid; dimness of vision with retrobulbar neuritis and central or paracentral scotomata were found. Wilkinson (1944; Wilkinson and King, 1944) reported from Hong Kong cases of nutritional amblyopia which was painless and without scotomata. Spillane (1945, 1947) investigated nutritional retrobulbar neuritis in German prisoners in the Middle East; the discs were normal, slightly pale temporally or slightly hyperaemic, and there were central scotomata. Other cases were studied in Allied prisoners in the Far East (e.g., Adolph *et al.*, 1944; Rich, 1946).

The factor or factors concerned in nutritional visual disturbances in man are not known; deficiency of some part of the vitamin B complex (other than niacin, riboflavin, and possibly thiamine) is the most probable cause. It is well known that pigeons with acute deficiency of thiamine are blind, and that, since this sign is cured in a matter of minutes by injection of thiamine, it presumably arises from a biochemical lesion in the brain with no structural lesion. The visual disturbances in man, however, differ in that they are not immediately cured by therapy, and their etiology is almost certainly different.

The testing of visual acuity is important when there is a recent history of *rapidly* failing vision, such as marked impairment occurring during a period of up to about two months; otherwise its only use for our present purposes is as an aid in the interpretation of certain tests of rod threshold of the dark-adapted eye.

3. Rod Threshold and Scotometry of the Dark-Adapted Eye

Various workers have shown that when human volunteers partake of a diet very low in carotenoids and in vitamin A, but apparently adequate in other nutrients and in aliments, a significant increase in the rod threshold of the dark-adapted eye occurs after a variable period. The period, it is claimed, may be one of days (Wald *et al.*, 1938; Wald and Steven, 1939; Drigalski, 1939; Hecht and Mandelbaum, 1940), of weeks (Booher

et al., 1939; Pett, 1939), or of many months (Wagner, 1940; Wald *et al.*, 1942; Brenner and Roberts, 1943; see also Steffens *et al.*, 1940, and Isaacs *et al.*, 1940). The differences in the time that elapses before the functional abnormality appears cannot at present be fully explained. In the spring of 1942 Steven (1942) and other members of the staff of the Oxford Nutrition Survey conducted an experiment in which nine students at Magdalen College and I partook of such a diet for periods varying from 38 to 150 days. Full curves of dark adaptation after preliminary exposure to bright light, and the threshold of the dark-adapted eye, were determined at intervals using a slightly modified Wald machine (Wald, 1941) which itself is a modification of Hecht's machine. That machine has the advantage that its readings are relatively independent of visual acuity and of intelligence, and the subject cannot cheat the machine. Therefore closely reproducible results are obtained in a normal subject provided there are no marked variations in pupil diameter. Of the ten subjects, seven showed a significant deterioration in rod threshold of the dark-adapted eye, and at the same time there had been a significant fall in the amount of vitamin A in serum; two subjects approached the level of clinical night-blindness, one after 54 days and the other after 87, the rises being 0.75 and 0.8 log units respectively. Three subjects were treated with massive doses of vitamin A (halibut liver oil being used), and the rod thresholds and serum vitamin A were found to be normal a few hours later. It is concluded that the impaired rod threshold was almost certainly caused by deficiency of vitamin A, although it is just possible that some other factor in the halibut-liver oil may have produced the response.

Subsequently the Medical Research Council planned a similar experiment on much more extensive lines (1945). The Oxford Nutrition Survey, amongst others, again measured the serum vitamin A and determined the rod threshold of the dark-adapted eye using the same machine. This time deficiency as judged by the two sets of values occurred in none of the 16 subjects within a year, and only in 3 of 11 subjects after 14 to 22 months.

Similar unexplained variations in time of depletion and in response to therapy had been encountered by Wald and by Hecht. Factors as yet unknown are undoubtedly concerned in the utilization of vitamin A and carotenoids in the body, and in the rate at which the body becomes depleted; this may be fast, as in hyperthermia, or slow. What is certain is that deficiency of vitamin A can cause an impaired rod threshold in the dark-adapted eye, and that under certain conditions this will revert to normal a few hours after administration of fish liver oil rich in vitamin A. We urgently need further information about the metabolism of

vitamin A and of carotenoids in the body. Recent work in the Netherlands and Germany has shown that in malnutrition there may be impaired utilization of carotenoids apparently caused by a dietary deficiency.

Deficiency of vitamin A is, of course, not the only cause of an impaired final rod threshold of the dark-adapted eye. If the threshold is being used in the assessment of human nutriture, the first essential is to employ a reliable instrument calibrated at frequent intervals. If an impaired threshold is found, the eye should be examined for other possible causes of this abnormality such as retinal pigment, or opacities of lens or cornea. If no such cause is discovered, therapy with vitamin A should be given and the rod threshold then retested. If a significant improvement occurs, it is very probable that the subject was deficient in vitamin A. The Oxford Nutrition Survey has used the following method for the routine testing of large numbers of persons. Persons enter the darkroom through a door with a light trap at intervals of about 3 minutes. They sit in absolute darkness for at least 20 minutes and then readings of the rod threshold are made with the modified Wald machine and continued until consistent results are obtained. If, after slit-lamp microscopical and ophthalmoscopical examinations of the eye have been made, there is no explanation of an impaired rod threshold, the subject is given 20,000 I.U. of vitamin A orally on each of 7 days and then retested. If a significant improvement is then found, the subject is believed to have been deficient in the vitamin, but if the threshold is stable to therapy, deficiency is not excluded: there are reasons as yet unknown why subjects deficient in vitamin A do not rapidly respond to such therapy, although there are of course factors other than deficiency of vitamin A that will cause an impaired threshold in absence of observed structural abnormalities of the eye.

In this work an area 2.5° in diameter located 6° above the fovea has been used. A similar area 20° below the fovea has also been tested in a large number of persons and there is a very high correlation between the two sets of readings. Livingston, however, has shown (1944) that scotometry of the dark-adapted eye would reveal a diminished field of rod function in subjects on a diet deficient in vitamin A and carotenoids before our testing at 6° above the fovea showed impaired function. It is probable therefore that scotometry detects deficiency at an earlier stage, but it is not practicable in routine assessments of nutriture.

4. Capillary Fragility

Measurements of capillary fragility have been made by modifications of two methods, that of Hecht (1907) in which a reduced pressure was applied to the outside of the capillaries, and that of Göthlin (1931) in

which an increased positive pressure was produced within them. The former method is the better and can conveniently be used on the capillaries of the skin by applying a cup, 1 sq. in. in area, to the front of the forearm just below the antecubital fossa and suddenly reducing the pressure by 200 mm. of mercury for 1 minute; the difference in the number of petechiae in the area before and after the reduction in pressure is estimated by viewing the area through a lens of about $\times 15$ diameters. Using this method we have found no correlation between capillary fragility and the amount of ascorbic acid in serum or in blood (thereby confirming earlier work of Degge, 1936; Liebmann *et al.*, 1938; Rapaport *et al.*, 1940; Scarborough and Gilchrist, 1944; and others) or in leucocytes or erythrocytes, or the fasting excretion of ascorbic acid, or the amount of a test-dose excreted. This merely supports the now general view that the earlier claims for the use of this test in the diagnosis of deficiency of ascorbic acid are erroneous. One striking demonstration of this error was that when Crandon had induced "frank scurvy" in himself by partaking of a diet deficient in ascorbic acid, both the increased and reduced-pressure tests gave negative results (Crandon *et al.*, 1940). In the last few years the work of Scarborough (1945), Bacharach *et al.* (1942) and others has indicated that in lower animals and possibly in man the test is useful in estimating deficiency of vitamin P.

The fragility of capillaries in the gums was possibly a more hopeful measurement, conveniently made by applying a reduced pressure to the gums at the base of the interdental papillae through an auriscope. Our results with this however have not been encouraging as an indication of deficiency of ascorbic acid.

5. Nerve Accommodation and Chronaxie

Various French authors have included measurement of chronaxie in an attempt to detect early neuromuscular disorders of nutritional origin. In work that was not carefully controlled, Elsom *et al.* (1940) claimed that 3 days after she started a diet low in the vitamin B complex, there was "diminution in electrical irritability of the peripheral nerves." According to Lecoq *et al.* (1946), "Dans l'appréciation des troubles neuromusculaires latents des avitaminoses, la méthode chronaximétrique constitue un test précieux qui permet de mettre en évidence la carence avant toutes manifestations cliniques." In lower animals deficiency of vitamin E increases chronaxie (Victor, 1934), but there is no evidence that this happens in man.

The measurement of nerve accommodation, rather than of chronaxie, would presumably find greater favor outside France, but insufficient work has been published to permit of an assessment of its use. In the

joint experiment already mentioned in which human volunteers were placed upon a diet deficient in thiamine for 86 days, some receiving supplements of thiamine daily, measurement of nerve accommodation at a time when a clinical examination was useless for distinguishing the two groups could produce a successful differentiation between deficient and control subjects in about 70% of them; estimations of thiamine in blood could at this time, as previously mentioned, differentiate the two groups with complete success. It is probable that the measurement of nerve accommodation in deficiency of thiamine will prove to be of value similar to that of the measurement of rod threshold in deficiency of vitamin A: impairment of function will be detected earlier than by the usual clinical examination, but nutritional deficiency will be only one possible cause of such impairment.

6. Ergometry, Endurance, and Ischemic Pain

Tests of physical fitness, of capacity for physical work or of fatigue have been used in the assessment of nutriture; they have been studied particularly in subjects placed experimentally upon deficient diets. In such work reliance can seldom be placed upon the reports of the subjects themselves or the subjective impressions of observers. Therefore carefully controlled tests have been introduced, particularly by Keys in Minneapolis and Johnson in Boston.

The performance of work by muscles is dependent in part upon the supply of aliments for yielding energy by metabolic changes, the supply of nutrients that take part in such changes, and the maintenance of homeostasis by counteracting chemical and physical processes resulting from such changes. Care, however, must be used in interpreting results: if administration of disodium hydrogen phosphate is found to diminish muscular fatigue or increase performance of work, it would not necessarily be correct to assume that the person was previously deficient in phosphate. Further, the performance of muscular work and the onset of subjective fatigue are greatly affected by motivation and by training. There is no doubt that both of those are affected in severe undernourishment, in anemia, in beriberi, in pellagra, and in scurvy; the problem is to find whether malnutriture is limiting performance of muscular work or increasing fatigue in an individual or group of persons. No single test is likely to achieve this since individual variation is so great, and therefore recourse must be made to comparison of groups, or to supplementation of the diet or, if the particular problem permits, to tests made on subjects placed upon deficient diets.

Since "endurance is apt to be a quick and sensitive indicator of nutritional inadequacy" (Keys, 1946), particularly of undernourishment,

tests of endurance are likely to be useful in nutritional surveys. But in these it is not easy to employ the type that is used in experimental laboratory research. Milligan (cf. Milligan and Lewis-Faning, 1942) introduced the bar test for children: the child hangs by the arms for as long as he will from a horizontal bar; the time is recorded and this or the product of multiplying it by body-weight is used as a measure of nutriture. Unfortunately motivation is a very important factor in a test such as this with children: if two or more children hang from the bar simultaneously, they will tend to be much "better nourished" than if they hang singly. When we applied this test to undernourished boys in Holland, we obtained shorter times than Milligan got with English boys; but it is doubtful to what extent the values under the different conditions obtaining in the two countries can be compared. Any test of endurance should test strength and motivation to as slight degrees as possible.

Though certain claims to the contrary have been made on somewhat inadequate grounds, the evidence is that no gain in fitness of adults occurs when vitamin supplements are added to a good American diet (Keys and Henschel, 1942; Foltz *et al.*, 1942; Ruffin and Cayer, 1944; Archdeacon and Murlin, 1944; Borsook *et al.*, 1946). Similar results were obtained in Britain during the war (Bransby *et al.*, 1944), but Kruse *et al.* (1944) have pointed out that under the conditions used the length of the study, which would correspond to 10 or 11 days in the life of a rat, might not be expected to permit of demonstrable improvement.

It might be supposed that vitamins of the B complex would play the most prominent part amongst the nutrients in affecting fitness or fatigue, since certain of them have been proved to take part in metabolic changes that yield energy. The Boston workers have claimed (Egafia *et al.*, 1942; Johnson *et al.*, 1942) that a diet deficient in vitamins of the B complex produces physical deterioration in sedentary men within four weeks and marked reduction in capacity for work in men doing hard work within a few days; but Keys (1943a) has pointed out that "after careful consideration these claims must be rejected in toto." In controlled work Keys and his colleagues found "no effect whatsoever of a very severe restriction in B vitamins coupled with hard work when this is carried on for a matter of a couple of weeks" (Keys, 1943b); the first notable effects were anorexia and mental depression, and some of the subsequent loss of fitness may have been due to undernourishment and tended to be corrected by thiamine alone (Keys *et al.*, 1945). On diets supplying 34 to 40% of the N.R.C.'s allowances of the major B vitamins, there was no observed loss in physical or mental capacity within at least 6 months (Keys *et al.*, 1945; Cogswell *et al.*, 1946). However, Barborka *et al.*

(1943), in well controlled work on four subjects, claimed that a diet low in thiamine (0.65 mg. daily) and riboflavin (0.94 mg. daily) "produced a definite decrease in work output shortly after it was begun"; and Archdeacon and Murlin (1944) obtained on a diet low in B complex vitamins greatly decreased muscular endurance that was markedly improved by thiamine but not by riboflavin. There are many claims (Csik and Bencsik, 1927; Morell, 1940; Gounelle, 1940; McCormick, 1940; Droese, 1941; Rosenbaum *et al.*, 1942) that the performance of work by individuals who show no signs of deficiency is increased by administration of vitamins of the B complex, but these have not been properly substantiated. A diet low in riboflavin was found by Keys *et al.* (1944) to produce no change in fitness in 6 months.

Since the onset of pain in a group of muscles exercised under controlled conditions is highly reproducible (Lewis *et al.*, 1929-31), and since this pain is presumably caused by the accumulation of some metabolite, we have tested the effect upon the onset of ischemic pain of injections of thiamine in normal subjects, and of experimentally induced deficiency of thiamine. Neither excess of thiamine nor deficiency influenced the onset of ischemic pain under the conditions of our tests.

7. *Dynamometry*

The force exerted under given conditions by groups of muscles has been measured by certain workers in the belief that this measurement would assist in the assessment of nutriture. A dynamometer that measures lumbar pull has been most frequently used. There is, however, enormous variation in strength between individuals; therefore cross-sectional studies are not likely to prove of value. And longitudinal studies when subjects have been placed upon deficient diets have shown that strength is very resistant to brief periods of deprivation: Brozek *et al.* (1946) found only a slight deterioration in strength in acute deprivation of vitamins of the B complex; and Keys and his colleagues also found that strength was much less affected than was endurance by undernourishment, although obviously strength is reduced when there is atrophy of muscles.

In nutritional surveys it would seem that no useful purpose is served by measurements of strength.

8. *Cardiovascular and Respiratory Function*

In undernourishment there is sinus bradycardia which may produce pulse rates below 40 beats a minute, or even below 30. There is also a fall in both systolic and diastolic blood-pressures, and an increase in circulation time. In deficiency of thiamine, on the other hand, there is

usually tachycardia, low diastolic pressure, and a decrease in circulation time with peripheral vasodilatation.

In the assessment of nutriture measurements of pulse rate and blood pressure are of value; measurements of vital capacity or of breath holding are worthless. Elaborate tests of cardiovascular or respiratory function, such as the pulse rate after standard exercise, are not usually practical.

9. Psychometric Tests and Coordination

As a result of his studies on acute deficiency of vitamins of the B complex, Keys (1947) concludes that intellectual capacity and the special senses are remarkably resistant, and psychomotor functions like neuromuscular coordination and speed are less affected than endurance. Coordination and steadiness greatly deteriorate in brief deficiency of aliments (Taylor *et al.*, 1945) or in prolonged semistarvation; whereas fasting for a few days produces a far greater decline in coordination than in strength, prolonged semistarvation produces greater losses in strength than in coordination (Keys, 1946).

Intellective functions are relatively resistant to nutritional deficiencies, although mental derangement occurs late in pellagra. As Keys (1946) points out, the claims of Harrell (1943, 1946) regarding the effect of added thiamine on learning in children are unacceptable.

V. SOMATOMETRIC ASSESSMENT

It is obvious that if there is an inadequate supply of the substances needed for the formation of new tissue during body growth, this will be limited; limitation may also occur if nutriture is disturbed by deficiency of other aliments or nutrients. This simple fact has been appreciated for a long time, and therefore much attention has been paid by nutritionists to the growth of children. But insufficient thought has been given to the most desirable rate of growth, which is not necessarily the maximum rate. We can make a boy of 12 years taller and heavier than he would otherwise be by injecting anterior pituitary lobe extract; alternatively we can make him heavier and probably taller by superalimentation. There is indeed a tendency amongst nutritionists to regard the child of perfect nutriture as placid, rotund, red faced, and seated in contented contemplation of its folds of flesh. During the past several years there has been a marked increase in the rate of growth of children, although in England and the U.S. the adult male height has remained unchanged. It has not been shown that this increase in rate is necessarily advantageous; indeed it may be undesirable since the long time taken to reach maturity is characteristic of the human genus. It is known from the work of Wolbach that in young rats and guinea-pigs fed on diets containing large

amounts of vitamin A there is a great acceleration of bone growth so that "it is possible to get the equivalent of a year's growth in a ten to fifteen day period" (Wolbach, 1945); the new bone is inadequately calcified and fractures easily. But a great acceleration of growth of this type seems to be the goal of many nutritionists despite its inconveniences.

The factors that condition growth include the following: heredity (including race and sex) and somatotype; birth conditions; age; geographical and temporal circumstances; activity; illness; obesity; nutrition. The use of some body measurement, or measurements, dependent on growth for the cross-sectional assessment of nutriture in an individual is therefore complex. In fact the unknowns are so many that the attempt is useless. A child with scurvy may have normal body measurements; rickets is a disease of growth, and athreptic infants that are not growing tend not to develop rickets; persons with famine edema may be statistically normal in weight even when allowance is made for the extra fluid. Recognition of the uselessness of sex-age-height-weight tables led to various other body measurements and a variety of indices being introduced. For instance, W/H^3 was used by Buffon in 1829, by Quetelet in 1836 and by Röhrer in 1908; other W/H indices were used by Tuxford (1917). Stem length was related to weight in von Pirquet's "Pelidisi" index, and to erect height in Manouvrier's index. Such cross-sectional data on single individuals are worthless.

After trying unsuccessfully to assess nutriture by weight, Oeder (1910) used the thickness of skin and subcutaneous tissue over the abdomen. Kornfeld and Schüller (1930) chose four sites (chest, abdomen, back and cheek). Mitchell (1932) used the excellent regression equations derived by Franzen (1929). Believing that the thickness over the arm is less affected by somatotype than that over the abdomen, we take the thickness at constant pressure of a fold of skin and subcutaneous tissue measured longitudinally over the top of biceps and midway down the back of the forearm; apart from depending on somatotype, the measurement is affected by age and sex, but it gives an indication of "thinness" which may be relevant mainly to aliment nutriture. It is obvious, however, that the athlete will tend to be "thinner" by this criterion when in training than when he is engaged in sedentary pursuits.

Somatometric methods can be very valuable, indeed, essential, to certain longitudinal studies, but these are not the main concern of this article. In our surveys in Germany we find it convenient and profitable to follow monthly the changes in weight of individuals and also of random samples of the population. For adults, the results are most easily computed and compared with other groups if the weights are first corrected for height. (It would be more satisfactory in theory to correct

weight for stem length.) But even in longitudinal studies of the body weight of individual adults, severe extraneous factors interfere. Such are the customary seasonal variations in nude weight, the greater seasonal variations in clothing if worn for weighing, and variations in the fluid content of the body (as in famine edema). In children there is the additional complication of growth spurts. It is well known that there are two accelerations in growth: the mid-growth spurt occurs from about the ages of $5\frac{1}{2}$ to $7\frac{1}{2}$ years in both sexes and takes place mainly by accelerated increases in girths; the adolescent spurt occurs in well-nourished North American girls from about 9 to 13 years of age and in boys about 2 years later, and is mainly accelerated increases in stem length. Unfortunately there are variations in the age of onset and in the duration of the spurts, which complicate a simple grid method of comparing actual growth with "normal" growth as is attempted by Wetzel (1941, 1943). Growth of nails (le Gros Clark and Buxton, 1938) and growth of hair are undoubtedly affected by nutrition, but have not been studied adequately.

VI. CLINICAL ASSESSMENT

1. *Mortality and Vital Statistics*

A physical examination is essential in the proper assessment of nutriture. But unfortunately clinical findings are harder to interpret and have been subjected to more fallacious interpretation, than any other group of data that we are discussing.

In comparing the nutriture of different populations, statistics of mortality have been used. As Jolliffe and Most (1943) point out, the usual mortality statistics give a very inadequate indication of the incidence of severe nutritional diseases. Deaths from certain diseases that are influenced by nutrition, such as pulmonary tuberculosis, or morbidity statistics concerning the incidence of such diseases are of some interest but are usually very difficult to interpret because of variations in other relevant factors. For instance, an apparent increase in the incidence of pulmonary tuberculosis (judged by the present returns of "active" cases or of deaths from the disease) in the British Zone of Germany as compared with 1939 might be due to a variety of causes including different criteria used in the returns, overcrowding, inadequate heating, or malnutrition.

The difficulty of interpreting certain statistics is also shown by studies of the effect of nutrition on complications of human pregnancy, childbirth, and infancy. This effect has been studied experimentally by three groups of workers: the National Birthday Trust Fund (Williams, 1937a, b, 1938, 1939; Balfour, 1938, 1944); the Toronto group (Ebbs *et al.*,

1941, 1942a, b); and the People's League of Health (1942). Although there are statistical objections to some of this work, the general results are impressive; a review of this work cannot be undertaken here. Baird (1945) has pointed out the importance of nutrition in prematurity and stillbirths, and Sutherland (1946) in the latter. Smith (1947a, b), however, could find no effect of the Dutch famine upon stillbirths or neonatal mortality, and no increase in toxemia.

2. Methods of Examination

An important feature of the physical examination is the differentiation of structural abnormalities that can be inspected. For this purpose various aids have been introduced. A loupe is of course customarily used by dermatologists and ophthalmologists. A very useful instrument for examining the skin and tongue is the type of illuminated magnifier used by thread provers and having a magnification of 10 or 20 diameters. For instance, the papillae of the tongue of a group of persons may be rapidly inspected with such an instrument by inserting microscope slides between the instrument and the tongue.

For higher magnification, the biomicroscope is used. We have modified an ordinary type of slit-lamp microscope so that it can be tilted downwards to view from above structures such as the tongue. The skin over the triceps area is conveniently inspected with any instrument if the patient stands with his back to the observer and rests his elbow in the chin-rest. For inspection of the limbus of the eye, the slit should be opened slightly, and the vessels examined by reflected light. This is greatly helped by the use of polarized light, produced by placing a disc of polaroid over the lens that focuses the slit. Plane polarized light makes the definition of the scleral-conjunctival margin very sharp, and also assists the appreciation of conjunctival opacity; the use of a second polarizer on the objective of the microscope enhances the effect but makes the retroillumination of the vessels too dim.

The interpretation of abnormalities of the limbus, together with that of other clinical signs, will be discussed below. Because of the importance of therapeutic trials in the interpretation of such signs, a note concerning them may be inserted here from our instructions for the use of teams in Germany.

Therapeutic Trials. It is obvious that the mere listing of the incidence of certain clinical signs (such as magenta tongues or folliculosis or gingivitis or pallor) is not of practical use unless some estimate of the etiology can be made. It is not only unscientific but actually misleading to attribute such signs to deficiency of particular nutrients unless evidence is available. Biochemical estimations, and even dietary investigations,

help in this, but when practicable therapeutic trials should be instituted. A group of subjects showing the lesion is selected and carefully examined, if possible an objective record of the lesion being obtained. The subjects are divided into two subgroups, one being given the therapeutic substance and the other no therapy (or preferably a placebo). The subjects are examined again after a suitable interval and at this examination it is essential that the clinician should not know which of the subjects are test and which controls. After the findings have been recorded on each subject, the results can be assessed. It should of course be pointed out to the subjects that something wrong with their health may have been found and that the treatment is being instituted to show whether this is so; at the re-examination it must be clearly established that the therapy has been followed, and the best indication of this is often afforded by a repeat biochemical examination.

When such therapeutic trials are carried out, and on other occasions, it is useful to make an objective record of the lesion. Several groups of workers have used skinprints or tongueprints (Sinclair, 1945). Excellent cameras have become available for making records, but a disastrous tendency has arisen to use them as a substitute for, rather than as an aid to, the clinician's examination. The RCAF ophthalmic camera (Tisdall *et al.*, 1943) is perhaps the best of such machines, but I have seen a print from it misinterpreted: in a survey in Northern Canada I had examined biomicroscopically the limbuses of the eyes of a Canadian Indian and pronounced them normal; but the dots of pigment that outlined the scleral digitations in this dark-skinned individual were interpreted in a photograph as being erythrocytes in abnormal limbal vessels and the individual was therefore pronounced by another observer to be deficient in riboflavin.

Whenever possible, of course, actual measurements should be made in the course of the examination, rather than qualitative observations. This is particularly important when different types of population are being examined because of the extreme difficulty of maintaining the same criteria: if a sign is found to be common in a particular population, but was rare in one previously examined, there is a tendency to alter one's criteria and grade grosser degrees of the sign.

Many signs can be quantitatively recorded if this is thought desirable. For instance, gingival tenderness can be measured by pressure with a blunt probe. It is easy to construct a spring-loaded probe that will give measurable pressure. The normal gum papillae should withstand a pressure of 1 kg. on 7 sq. mm. (14 atmospheres) without pain or bleeding. Such measurements are often made by physiologists in the study of the normal but rarely by clinicians in their study of the abnormal.

For instance, Ryle (1947), in Oxford, has classed the strength of the knee jerk and the heart sounds as "immeasurables"; the former was measured by Franklin (1938) in Oxford, and the latter are frequently recorded by physiologists using a stethocardiograph.

It is possible to examine briefly in nutritional surveys for public health purposes large numbers of subjects in a short time if a routine is adopted. Our usual method is as follows: Males and children up to the age of 12 are examined unclothed to the waist and without shoes and socks; females (except girls under 12) are examined without shoes and stockings and with the whole of one arm bare. While the subject is sitting, the lips, teeth and gums, and tongue are examined; this is followed by the pulse, skin of the arms, muscle irritability, thickness of skin and subcutaneous tissue, vibration sense, edema, skin of the legs, calf tenderness, and jerks. The subject then stands and the following examinations are made: heart, lungs, and skin of the back of the chest and triceps (abdomen for enlarged liver or spleen if these are to be felt in children in the standing position), eyes and skin of face, posture, thinness. Then follow any special biomicroscopical examinations. Finally, any comments are made about the mentality of the subject and his nutriture, and then the diagnosis is recorded.

3. General Assessment by Inspection

At the beginning of this century attention began to be paid in various countries to the assessment of nutriture by a rapid inspection of (usually) children. In England, for instance, the medical inspection of school-children was introduced by the Board of Education in 1907, and in this the assessment of nutriture played a part. Such examinations usually assessed physical state, of which health is one component; and nutriture is a component of health: it is not surprising therefore that they were often only remotely connected with nutriture. When nutriture was separated from the other components, it was often identified with growth in relation to age, or with amount of subcutaneous fat (Koppe, 1905) with or without extent of muscular development (Hogarth, 1909), or pallor (Gastpar, 1908). When nutriture was not so distinguished, it often became synonymous with physical state, as in the Dunfermline system (Mackenzie, 1913). In this, which formed the pattern of much subsequent work, the following were taken into account: height and weight in relation to age; general appearance; mucous membranes, skin and subcutaneous tissue; muscular tone and development; facial expression, posture, movements, voice, interest and attention.

After the First World War, von Pirquet with Nobel and others intro-

duced the Sacratama index which rated the following: the blood content of the skin, the amount of subcutaneous fat, the skin tension determined by the water content of the subcutaneous tissue, and the condition of the muscles. The Berlin conference of the League of Nations recommended (1933) that a "general impression" of the condition and constitution of persons examined should be recorded, and for this purpose they recommended von Pirquet's methods.

In 1934 the Board of Education in England issued instructions (Admin. Mem. No. 124) for the use of School Medical Officers in their assessment of nutriture which was not to be based solely on height and weight. Children were put into four classes (excellent, normal, slightly subnormal, bad) in an attempt to determine undernourishment on the basis of: general appearance; expression; posture; tone and function of the muscular system; condition of the skin and hair; color of the mucous membranes; amount of subcutaneous fat; teeth; presence of any defect or deformity. In 1937 the Advisory Committee on Nutrition of the Ministry of Health concluded that they could not recommend as reliable any of the known methods of assessing nutriture, but they considered the above method of the Board of Education to be the most promising; the Committee believed "that research should be continued to establish, if possible, a reliable test or group of tests for the assessment of the state of nutrition." There are three pertinent questions regarding the Board's method: first, whether it in fact assesses general nutriture rather than muscular development and physique; secondly, whether experienced School Medical Officers are consistent one with another in their grading of children; thirdly, whether one such experienced doctor is consistent with himself on different occasions. The last two questions were excellently investigated by Jones (1938) who obtained very definitely negative answers to them. For instance, in one experiment in which five doctors examined the same 193 boys and re-examined them a week later, "on both occasions in more than half the cases, the doctors failed to agree over the crucial question of whether a boy is satisfactorily nourished or not," and one doctor found 1.5% to be subnormal or bad whereas another doctor put 47% in these classes. And the doctors' results at the second examination were not consistent with their results at the first. Jones's correlations between the ratings given by the doctors and various somatometric indices make it extremely probable that the doctors were strongly influenced by the height and weight of the children. Jones found that "On the average, the experienced School Medical Officer picks out two-thirds, and [Tuxford's height-weight] index three-quarters, of the boys assessed subnormal by two or more doctors." It seems that the Board of Education assessment has no advantages, and certain disad-

vantages, over simple records of height and weight, and these cannot alone be satisfactorily used to assess general nutriture.

Recently some results of surveys made by the Ministry of Health clinical team have been published (Adcock *et al.*, 1947); in this work five clinicians have at different times taken part. This paper contains certain errors, and I have mentioned elsewhere some that occur in it (Sinclair, 1947d). The authors have used two types of clinical methods. First, the clinician placed the subject into one of three grades—"good," "fair," "poor"—according to his "general state of nutrition." Secondly, the presence of 29 "clinical signs" was recorded. In this paper the findings on 3351 children aged 8–15 years and on 3326 adults are analyzed statistically to ascertain whether any relationship exists between the incidence of the "clinical signs" singly or collectively, and "the clinician's nutritional grade." The incidence of each clinical sign is also compared with "the nutritional state as a combination of all the clinical signs." The authors conclude that "nutritional assessment as at present understood is determined mainly by the value placed on the grading criteria . . . and to a negligible extent or not at all by the presence or absence of clinical signs." The "clinical signs" (which are sometimes also called "nutritional signs") include "dandruff," "pityriasis," "acne," "ichthyosis," "pinguecula," "oedema"; it is obvious therefore that they include many signs that are not nutritional in origin, and the term "nutritional signs" should not be used for them, particularly as the authors conclude that the signs play little if any part in the "nutritional assessment as at present understood." The authors use for "the nutritional state as a combination of all the clinical signs" the term "the general nutritional factor"; from this it follows that an adolescent with a marked seborrheic diathesis will have a very poor "nutritional state," scoring under "seborrhea," "dandruff," "acne," and perhaps "pityriasis"; whereas an adolescent with gross undernourishment (thin, pale, wasted, with inelastic skin) will probably not have any "nutritional sign" recorded and therefore will be in an excellent "nutritional state." The authors state that the clinical signs were "not taken into account in grading the subjects according to their state of nutrition." Yet one such sign, edema, occurs also in the list of grading criteria. It is possible for a child to have scurvy, and have none of the grading criteria; yet it would be hard indeed for the clinician to put such a child in the class of "good nutrition." Further, the paper to which the authors refer for further details (Magee, 1944) specifically attributes the higher incidence of children graded "poor" in these surveys, as compared with the incidence in the examinations of the School Medical Service, to the "more elaborate methods of diagnosis used by the Ministry's observers" including the slit-lamp

microscope; therefore the clinical signs found in the eye with this instrument (e.g., corneal vascularization) were in fact used in assessing "nutritional grade" in contradistinction to the statement in the present paper. It is rather remarkable that corneal vascularization is apparently commoner than dandruff or acne in adolescents, or than pingueculae or pyorrhea in adults—the more remarkable as two of the clinicians, Drs. Sydenstricker and Stannus, have in their publications wisely adopted a conservative attitude about what constitutes invasion of the cornea. Since the authors find no correlation between the signs singly and "an internal factor (the general nutritional factor) responsible for the signs," they conclude that the clinical signs play a negligible or no part in the assessment of nutriture. This conclusion is clearly not justified since there is obviously no single internal factor responsible for the signs; and even if all of them were caused by nutritional deficiencies, the varied combinations of signs in different deficiencies would preclude such positive correlations. The authors find a high degree of correlation of these selected "grading criteria" with "nutritional assessment"; for instance, a child that is abnormally thin is likely to be graded "fair" or "poor," and not "good." From this the authors conclude "that the grading criteria are the main elements in the diagnosis of nutritional grade" (which is so by definition). It is apparent that the authors consider that the clinician usually regards a child with poor posture as being in a poor nutritional state, whereas a child with scurvy may well be regarded as being in a good nutritional state. We see but once more that this type of so-called nutritional assessment, whereby persons are placed in categories on the basis of posture and expression and the like, is worthless as an assessment of general nutriture. And we also find in this study no reason for doubting the value of recording true clinical signs of malnutriture.

Unfortunately the quest for such true clinical signs is made difficult by their unspecificity. And therefore a large part of this article must be devoted to a discussion of them.

4. Symptoms

A medical history is essential in the proper assessment of nutriture. Complaints volunteered by the person should first be investigated and past history obtained with particular reference to illnesses in the past year. Then information regarding certain specific symptoms should be sought. The most useful are: change in body weight, muscle pain or cramps including pain on rising from a chair, sore tongue, sore eyes, rapidly failing vision, paresthesia, weakness or fatigue, nycturia, diarrhea; information about menstruation and lactation should be obtained when relevant. We have found a history of edema almost useless: when asked

the usual questions some persons will give a negative answer even when they have marked pitting edema, whereas others will with no justification complain for instance of tight shoes. In general, symptoms must be accepted with great caution. One person will suffer in stoic silence grave insults to the body; another will whine about trivial or even imaginary ills.

5. Signs

It is perhaps fortunate that epithelial tissues are affected more than are mesenchymal by nutritional deficiency, and of the epithelial tissues the epidermis, the hair and the cornea are affected more than are others. Since these three are easily inspected, the elucidation of clinical signs is assisted. It might of course be supposed that the ease of inspection is the cause of the more numerous descriptions of lesions in these tissues; but histological studies of organs in deficiencies in both man and other animals have made this unlikely.

Skin, Hair, and Nails. In the assessment of nutriture of groups of the population the following should be recorded regarding the skin and its appendages.

i. Extent of examination. (It is often forgotten that the larger the area of skin inspected, the greater the chance of finding abnormalities; further, the depigmentation of kwashiorkor occurs around the anus, folliculosis commonly upon the buttocks, and the dermatitis of ariboflavinosis around the external genital organs. Therefore if populations or individuals are to be compared, the extent of the examination must be the same.)

ii. Pallor, suborbital pigmentation, generalized pigmentation (and depigmentation), erythema, ecchymoses, perifollicular petechiae.

iii. Eczema, "sores" and unhealed lesions, nutritional seborrhea, pellagrous dermatosis, crackling, pityriasis sicca, generalized xeroderma, desquamation.

iv. Diminished elasticity, atrophy, thickening, folliculosis, follicular hyperkeratosis.

v. Folliculitis, infestations.

vi. Hypochromotrichia. Other hair abnormality.

vii. Onychodystrophy.

Deficiencies of at least 11 nutrients probably cause alterations in the epidermis in mammals; and because of the confusion regarding such lesions in man, those occurring in the early stages of deficiency in the rat will be briefly summarized following the excellent studies of Sullivan and others.

In the rat, deficiency of vitamin A (Sullivan and Evans, 1945) causes

keratinization of the epithelium and hair follicles with no changes in the corium and sebaceous glands. Deficiency of linoleic or arachidonic acid (Williamson, 1941) causes hyperkeratosis of the epithelium with necrosis of the tip of the tail and scaliness of the dorsum of the paws. Deficiency of riboflavin (Sullivan and Nicholls, 1941) causes atrophy of the skin and appendages with alopecia. Hyperkeratosis and acanthosis are caused by deficiencies of pyridoxine (Sullivan and Nicholls, 1940), pantothenic acid (Sullivan and Nicholls, 1942a) or biotin (Sullivan and Nicholls, 1942b); in the first, there is also marked hyperemia and edema ("rat acrodynia"), in the second there is also dilatation of the hair follicles accompanied by greying of the fur and some alopecia, and in deficiency of biotin there is also alopecia. Greying of the fur is also caused by deficiency of *p*-aminobenzoic acid (Ansbacher, 1941). Deficiency of magnesium causes hyperemia of the corium, particularly of the ears and paws with hyperkeratosis later (Sullivan and Evans, 1944), and deficiency of zinc causes hyperkeratosis with atrophy of the hair follicles and later hyperemia of the corium (Follis *et al.*, 1941). This, however, is in rats. In mice, deficiency of inositol causes alopecia (Woolley, 1941). These results on lower animals cannot necessarily be applied to man.

The commonest change in lower animals is hyperkeratosis, often with acanthosis and rarely with parakeratosis. In man there are two broad types of hyperkeratosis caused by nutritional deficiency: in one it is limited to the hair follicles, and in the other it occurs in areas subjected to trauma whether by irradiation or pressure. The former includes different types of lesion as previously pointed out (Sinclair, 1945). One type, folliculosis, consists of hyperkeratosis surrounding the mouth of the follicle and forming a plaque that covers the mouth and imprisons the hair which remains coiled beneath it or may burst forth carrying the plaque and superficially resembling a spine; it is found particularly over the buttocks, thighs, calves, and upper arms. Folliculosis can undoubtedly be caused by deficiency of ascorbic acid; many of the early accounts of scurvy mention it, and proof was afforded by Crandon (Crandon *et al.*, 1940). Follicular hyperkeratosis, in which a spine often projects from the follicle alongside the hair, is alleged by many authors to be caused by deficiency of vitamin A and by others to be pathognomonic of such deficiency. The first claim is probably true but lacks rigid proof; the second is false. Hyperkeratotic papules without a spine are common at puberty, particularly in obese people; in obese women they tend to persist and often are erythematous. An important point is that in these cases, in which there is no dietary deficiency, there is usually no generalized xerosis of the skin. This contrasts strongly with a very distinct form of follicular hyperkeratosis that is found in malnutrition and which

resembles goose-skin due to cold even in having erect hairs and is accompanied by generalized xerosis; it may perhaps be caused by deficiency of vitamin A or of unsaturated fatty acids (and these are almost always administered with so-called "specific" therapy with vitamin A since they are contained in fish-liver oils and arachis oil), and it can certainly be caused by deficiency of aliments.

The second form of hyperkeratosis, namely, the generalized type, can be caused by chronic deficiency of niacin. The thickened skin, which may not be pigmented, is found most frequently over bony prominences and also the palms of the hands and soles of the feet.

Erythema of the epidermis is another striking manifestation of nutritional deficiency in lower animals, and it occurs in certain deficiencies in man. Whether the primary lesion is in the cells of the epidermis or in the cells of the minute vessels supplying it is a question that need not now detain us, but passing reference will be made to it later. In acute deficiency of niacin erythema, which occurs in areas subjected to mild trauma, is the first visible change; there is dilatation of the vessels in the superficial corium and hyperkeratosis with parakeratosis in the overlying epithelium; the sebaceous glands atrophy. Other changes occur later, giving for instance the cracked pigmented type of skin found particularly on the shins. Two other changes that occur particularly over the shins are a fine branny desquamation and thin shiny atrophic skin; the former which we saw frequently in Germany after the war occurs not uncommonly in malnutrition and can also be caused in the exceptional circumstances of biotin deficiency (Sydenstricker *et al.*, 1942). Dry scaly erythematous patches, pityriasis sicca, occur on the face of children particularly in the areas where secretions such as tears and saliva tend to accumulate; they may be caused by nutritional deficiency or may be related to deficiency of soap. Neither acrodermatitis nor acne rosacea is caused by nutritional deficiency. Erythema is also part of the seborrheic dermatitis found in deficiency of riboflavin; this affects particular regions such as the hair margin on the head, the malar eminences, and the scrotum, and also mucocutaneous junctions. In the rat deficiency of riboflavin causes the sebaceous glands at first to hypertrophy and then to atrophy (but in the mouse there is no change in them); it seems that in man also there is an alteration in these glands. Hemorrhages in the epidermis occur in deficiencies of ascorbic acid and of vitamin K. In the former, characteristic perifollicular petechiae are produced; in the latter, ecchymoses. It seems to be not uncommon for bug-bites to be attributed to deficiency of ascorbic acid; and a lady with perifollicular erythema and folliculosis was once demonstrated to me by a distinguished professor as a case of scurvy. Pallor of the skin and mucous

membranes occurs, of course, in nutritional anemia, and pigmentation is frequently found in deficiency of alimENTS. In the famine in the Netherlands in 1945 such pigmentation was not uncommon, and pigmentation followed by vitiligo-like depigmentation was also seen; the latter usually had the very characteristic depigmentation around the anus such as is found in kwashiorkor.

In this disease greying of the hair is found, and such was also encountered in the Netherlands. By analogy with the fur of rats, deficiency of pantothenic acid or of *p*-aminobenzoic acid might be the cause, but such analogies are very dangerous. In deficiency of alimENTS a downy hirsutism may appear particularly on the face. 'Staring' hair has been mentioned as being of diagnostic importance in assessing nutriture, but it is very doubtful whether it can be properly appraised even in children; Adamson *et al.* (1945) found it in a tenth of all subjects examined in Newfoundland, but how it was appraised in men over 60 years of age is not mentioned. During our study of the famine in the Netherlands an experienced Dutch pediatrician selected for us a boy with 'staring' hair; he was later presented again to the pediatrician after his hair has been brushed, and another boy not previously selected whose hair was later ruffled was also presented; the 'staring' quality was now found to have passed from one to the other. Hair is easily collected in nutritional surveys, and its chemical analysis might well repay study.

The relation of nutritional disturbances to onychodystrophies has not been adequately investigated. Koilonychia can be caused by deficiency of iron and apparently also by deficiency of calcium (or vitamin D). Atrophic nails, thin and brittle, can be caused by malnutrition; and longitudinal ridges, which may be of no diagnostic significance, can apparently be caused by chronic avitaminosis (White, 1934). Leuconychia has no significance in the assessment of nutriture.

Tongue. The tongue is interesting in that it has been extensively studied in nutritional disorders in man, but hardly at all in lower animals. And the former is being assisted by adequate glossoscopy and by recording through color photographs or tongueprints.

It is well-known that atrophy of the papillae, both filiform and fungiform, can be caused by deficiency of iron, and they will regenerate within a few days after iron therapy; this result is too rapid to be due to an increase in hemoglobin. The smooth tongue produced by deficiency of the hemopoietic factor, as in pernicious anaemia, regenerates quickly when treated with liver preparations, and in this case the filiform papillae reappear first (Frantzell *et al.*, 1945).

The tongue in pellagra has received much attention, particularly from the pen of Kruse in a preliminary report (1942b). His detailed

descriptions of the four acute and five chronic stages of aniacinosis, each with three degrees of intensity, is challenging; and the full report of his observations is awaited. At the time of the preliminary report 5 years ago, in 3 of 15 persons given specific therapy the lesions had "almost completely disappeared"; and this preliminary report, without discussing differential diagnosis, makes the sweeping and unacceptable claim that "From these lines of evidence, glossitis is seen to be a specific sign of pellagra and aniacinosis."

Whereas denudation of the epithelium of the papillae with an active circulation is the generally accepted picture in niacin deficiency, slight denudation with engorged dilated capillaries and vascular stasis with edema is found in deficiency of riboflavin (Sydenstricker, 1941, Kruse *et al.*, 1940). Though no doubt the smooth scarlet and the pebbled magenta tongues are very important and justify color-matching similar to that introduced by Lewis (1929) for the skin, their specificity remains to be proved. But there is no doubt that the epithelial cells of the tongue respond quickly to metabolic disturbances caused by malnutrition, and also regenerate with phenomenal speed when the disturbances are corrected. Further study would be well repaid. It must be remembered, however, that those cells are also very sensitive to trauma: we found in England during the war and in the famine in the Netherlands that an upper denture was the commonest single cause of denudation of the filiform papillae in adults (Sinclair, 1948b); and hot food or spices or excessive smoking (when this was possible in England) will produce bright red fungiform papillae. Fissures of the tongue are also deceptive because they can occur congenitally as in the familiar scrotal tongue.

Gums. Gingivitis is a classical feature of scurvy, and has been regarded almost without question as a specific lesion of deficiency of ascorbic acid. King (1943) and several others have claimed a connection between niacin and Vincent's gingivo-stomatitis, but this has been disputed by several authors.

Unfortunately most of the work on the relation between ascorbic acid and gingivitis will not bear a critical analysis. And seldom in nutritional surveys are reliable statistics regarding the incidence of gingivitis presented. It is usually forgotten that in edentulous persons the incidence of gingivitis is negligible, and therefore data about the presence of teeth must be recorded, since otherwise the apparent incidence of gingivitis will increase and then diminish with age; this elementary fact has usually been overlooked.

Kruse (1942c) has presented a preliminary report based on an examination of the gums macroscopically and biomicroscopically. He believes that "in all respects, therefore, examination of the gums forms a satis-

factory basis for appraisal of vitamin C status . . . [The changes in the gingival tissue] are specific and constant in occurrence. They are present in all states of avitaminosis C: they appear early, persist and reflect its course." Since in the persons examined by Kruse gross manifestations were readily seen in many, it is surprising that in Crandon no macroscopical changes were detected when he had clinical scurvy; it may even be doubted whether deficiency of ascorbic acid produces macroscopical changes in the gums in the absence of other factors such as trauma: constancy in occurrence of gingival changes is certainly doubtful. More important, Kruse's claim of specificity for the gingival lesions he describes cannot be accepted. Redness, swelling, and bleeding of the gums can be produced in monkeys by injections of estrogens (Ziskin, 1937), and in women tend to occur with menstruation and particularly with pregnancy. This has sometimes led to false claims about an increase of deficiency of ascorbic acid in women who are pregnant. Further, there is every reason to believe that the normal gum is pitted rather like orange-peel, and there is no justification for attributing this, as Kruse does, to the presence of atrophy caused by chronic deficiency of ascorbic acid. A group of distinguished nutritional experts who made a survey in Newfoundland (Adamson *et al.*, 1945) believe, with Kruse, that "The most frequent and reliable early clinical signs of vitamin C deficiency are to be seen in the gums." On the basis of macroscopical changes in the gums, they conclude: "One or more obvious signs of either acute or chronic gum involvement occurred in 69% of the population examined. . . . The clinical evidence indicates a high prevalence of a deficiency of ascorbic acid among the people of Newfoundland." Grave exception should be taken to these statements.

Certainly the color of the gums and the presence of swelling and also bleeding under defined conditions should be noted in assessing nutriture. But these signs may not be present in deficiency and most certainly are not pathognomonic of deficiency; and their incidence should be interpreted in association with relevant findings: these include the presence and state (including malposition) of the teeth; mouth breathing, heavy smoking and other forms of trauma; and pregnancy.

Teeth. In lower animals dentine is affected by deficiencies of calcium, phosphorus, vitamin D, and ascorbic acid; enamel is affected by deficiencies of vitamin A and magnesium, and by excess of vitamin A or fluorine. For reasons already mentioned, the presence or absence of teeth and of an upper prosthesis must be recorded, and it is usual to record the incidence of caries; if this is done, the presence of fluorosis should also be noted.

The aetiology of caries cannot here be discussed, but the relevance

of the incidence of caries to an assessment of present nutriture is highly doubtful. The relation of deficiency of fluorine to dental caries is beautifully shown by McClendon's studies (McClendon, 1944; McClendon and Foster, 1945) on the production of caries in rats on fluorine-free diets, and by studies in children of applying sodium fluoride to certain teeth (Knutson and Armstrong, 1945); if caries is recorded, mottling of the enamel should be included.

Mucous Membranes and Mucocutaneous Junctions. The buccal mucosa, the lips and those mucocutaneous junctions that are readily inspected, play a prominent part in assessment of nutriture. Cheilosis is a condition, described by Sebrell and Butler (1938, 1939), in which there is denudation of the epithelium of the lips along the line of closure, most commonly in the centre of the lower lip; the lip becomes abnormally red and may be sore. The condition occurs in deficiency of riboflavin but is not pathognomonic and can be caused by climatic exposure; Machella (1942) showed its nonspecificity, and Cayer *et al.* (1945) found deficiency of niacin and not of riboflavin was related to cheilosis in their patients. It differs, and should be distinguished, from angular stomatitis which consists of ridging and later fissures arising at the mucocutaneous junction at the corner of the mouth. Angular stomatitis also occurs in deficiency of riboflavin but is also not pathognomonic: it can be caused by trauma arising from an ill-fitting upper prosthesis. We use the term myceterosis for a similar condition arising at mucocutaneous junctions: the palpebral fissure, the nares, the external auditory meatus, the prepuce, vulva, and anus. Sydenstricker (1941), Mitra (1943), Stannus (1944) and many others have described it in deficiency of riboflavin, and it is certainly an important sign.

Three forms of stomatitis (acute catarrhal, aphthous, and Mikulicz's) tend to occur in malnutriture. Buccal denudation along the line of closure of the teeth has been attributed by Sandstead (1943) to deficiency of riboflavin, but this is certainly not a usual cause of this fairly common condition.

Eyes. In lower animals the cornea is affected by deficiencies of vitamin A, riboflavin, tryptophan, lysine, histidine, sodium, and zinc. The conjunctiva is affected by deficiencies of vitamin A, sodium, and perhaps riboflavin; the lens by deficiencies of calcium, riboflavin, and tryptophan; the retina by deficiency of vitamin A; and hemorrhages within the eye bulb occur in deficiency of choline and in hypervitaminosis A. Deficiency of vitamin A affects the lacrimal glands, of sodium the tarsal glands, and of pantothenic acid the Harderian glands. The relevance of these studies to man is doubtful. Deficiencies of the seven nutrients listed above as affecting the cornea all cause corneal vasculari-

zation in the rat; but the cornea of the rat in particular seems to be especially subject to vascularization, and other animals might well be studied.

The full and somewhat melancholy story of corneal vascularization in man cannot here be told. Following the work on rats that started with Day's observations in 1931, Sydenstricker, Kruse and colleagues (Kruse *et al.*, 1940; Sydenstricker *et al.*, 1940) described corneal vascularization in subjects made deficient in riboflavin. The authors regarded conjunctivitis as the primary step in the development of superficial keratitis in ariboflavinosis, and interstitial keratitis also resulted from such deficiency and could involve the whole depth of the cornea; a specific type of glossitis often occurred before other signs of ariboflavinosis were present (Kruse *et al.*, 1940), but later it was asserted that in this deficiency "the earliest and most constant finding is a superficial vascularization of the cornea" (Sydenstricker, 1941). Numerous subsequent papers have used corneal vascularization or circumcorneal injection as specific signs of deficiency of riboflavin; and at the other extreme Vail and Ascher (1942) have suggested that what Sydenstricker termed "corneal vascularization is nothing more than extensive engorgement of the preexisting limbal meshwork" except in cases of obvious corneal disease. Unfortunately two most important questions have been frequently overlooked: what is to be regarded as pathological vascularization of the limbus, and whether pathological vascularization is pathognomonic of deficiency of riboflavin. Everyone will, or should, agree that circumcorneal injection is not pathognomonic of deficiency: it is easily produced by rubbing the eye. The normal limbus, which has been excellently studied by Graves (1934), is supplied throughout its width with capillaries; this might be expected since the limbus is the whole zone in which the sclera and cornea overlap, and the sclera is vascular. In his early papers, however, Sydenstricker (1941) spoke of "the normal avascular zone between the plexus and the sclero-corneal junction"; later he regarded the limbus as vascular. The point is important because if we are to regard the limbus as normally avascular, as Kruse and others do, the incidence of corneal vascularization will be 100%, particularly if some substance such as dionine is used to dilate the temporarily occluded capillaries. It is not surprising, but depressing, to find therefore that in the survey conducted in Newfoundland by several distinguished experts (Adamson *et al.*, 1945), "100% showed some degree of vascularity of the cornea." During the war my own eyes were examined by seven experts in England (including Dr. Sydenstricker) and pronounced normal; after flying the Atlantic they were examined by seven experts in the U.S. and Canada and all seven said I had ariboflavinosis (according to Mr. Basil Graves, I have con-

centric collaterals as described by him (1934) in the normal limbus and by Vail and Ascher (1942) as occurring as a result of some "provoking cause"); but I had then taken riboflavin daily for three years and drunk beer, in moderation, for twenty-six years. This difference in interpretation is confusing. Obviously the extreme inner limit of the limbus must be defined: as mentioned elsewhere, polarized light assists this; and dionine helps in revealing the capillaries. But if capillaries overstep the limbus and invade the cornea superficially and fairly symmetrically and usually in both eyes, the condition may be due to deficiency of riboflavin or it may not. The dietary history, the presence of other signs of possible ariboflavinosis (so admirably described by Sydenstricker in various publications), the level of riboflavin in leucocytes or other tissues, and the response to specific and controlled therapy, these will decide the etiology of the pathological lesion and without at least one of them the decision cannot be made.

Since deficiency of certain amino acids has been found to produce corneal vascularization in the rat (Totter and Day, 1942; Albanese *et al.*, 1943; Sydenstricker *et al.*, 1946) we examined 155 persons attending a "polyclinic" for malnourished patients during the famine in the Netherlands; the incidence of corneal vascularisation was 7%, which is higher than that found in the same age groups in England.

Xerosis and keratomalacia are classical signs of deficiency of vitamin A (Pillat, 1931), and vascularization of the substantia propria occurs probably as a secondary phenomenon. It is customary to regard the changes in the corneal epithelial cells as the direct effect of deficiency of vitamin A upon them, but it is possible that part at least of the changes may be produced by deficient secretion of the ocular glands: in Sjögren's syndrome, keratitis occurs. Xerosis and keratomalacia are late changes in deficiency of vitamin A, and a characteristic feature is the painlessness of the condition.

Changes in the conjunctiva in nutritional deficiencies have achieved much prominence. Marked conjunctivitis, such as occurs in snow blindness, has been attributed to deficiency of riboflavin; and it certainly appears from the work of Sydenstricker that such deficiency is one cause of conjunctivitis. More important is deficiency of vitamin A. Kruse (1941) has presented a preliminary report of the conjunctival manifestations of avitaminosis A, which he found in 99% of the subjects he examined. Unfortunately no distinction is made in his description of conjunctival spots between pingueculae and true Bitot's spots. Pingueculae, of course, are caused by degenerative changes in the fibrous tissue in the substantia propria beneath the epithelium which is normal; Bitot's spots are quite different in appearance and are epithelial in origin. There

is no evidence that pingueculae or pterygia are nutritional in origin. Bitot's spots, which are very rare in temperate climates, can no doubt be caused by deficiency of vitamin A, and this is probably their commonest cause; but we have had the opportunity of studying cases that were not, and probably had not been, accompanied by such deficiency. Kruse suggests that xerosis conjunctivae probably precedes night blindness as an early sign of avitaminosis A. We have had the opportunity of repeatedly examining with a biomicroscope the eyes of volunteers placed upon diets deficient in carotenoids and vitamin A in two separate experiments: in neither experiment was any change in the conjunctival epithelium detected in any subject, and in both experiments the final rod threshold of the dark-adapted eye of some of the subjects rose significantly and reverted to normal after therapy with halibut-liver oil.

Changes in the lens occur in deficiencies of calcium (Meyer and Meyer, 1944), riboflavin (Day *et al.*, 1938) and tryptophan (Totter and Day, 1942; Buschke, 1943) in lower animals. In man, insufficient attention has been paid to structural changes in the lens in nutritional deficiencies, and such changes might well be important.

Glands. No discussion of nutritional deficiencies as affecting glands is possible here. Changes in the sebaceous glands occur in deficiencies of vitamin A and of riboflavin. Apart from the thyroid gland, in which epithelial hyperplasia is apparently produced by deficiency of iodine or excess of fluorine, few other glands are conveniently included in the assessment of nutriture.

Nervous System. Possible changes in the nervous system in deficiencies of thiamine, riboflavin, niacin, and copper cannot here be discussed, nor can the neurological conditions that arose as a result of wartime dietary restrictions; these have been admirably discussed by Grande and Peraita (1941), Denny-Brown (1947), and Spillane (1947). In a few experiments in which human volunteers have been placed upon a diet deficient in thiamine, polyneuritis has definitely been produced, but the accompanying lesion, presumably morphological as well as biochemical, has taken a very long time to respond to specific therapy (Williams *et al.*, 1943). Retrobulbar neuritis has been mentioned elsewhere in this article; the precise nutritional causes of spinal ataxia, spastic ataxia, spastic paraplegia, and "burning feet" are obscure but deficiency of the vitamin B₂ complex seems to be concerned.

In a rapid assessment of nutriture, the elicitation of knee- and ankle-jerks, of vibratory sense, and of muscular weakness (as, for instance, elicited by the squatting test) are of interest; but it is obvious that the presence of one or more of these signs must be regarded as an indication for a fuller examination. The Newfoundland survey (Adamson *et al.*,

1945) found one or more of these signs present in 6.8% of the population examined and "in view of this and of the numerous unsolicited complaints referable to the nervous system, it is likely that the thiamine status of a considerable proportion of the population is unsatisfactory"; "likely" is far too strong a word, and the statement elsewhere that "clinical signs which result from a lack of thiamine were encountered in 6.8% of the persons examined" is highly misleading.

Connective Tissue, Muscle, Bone and Cartilage. The relation of ascorbic acid to the differentiation of mesenchymal cells to form fibroblasts with production of collagen, and the relation of ascorbic acid and of calcium to the formation of intercellular cement substance, are complicated and mainly relevant here in consideration of the defects in wound healing that occur in deficiency of ascorbic acid.

Cartilage and bone are specialized connective tissues which are affected by deficiencies of calcium, phosphorus, vitamins D and A, and manganese. Deficiency of vitamin A slows endochondral bone formation, decreasing osteoblastic activity, and growth of cartilage cells; and if in excess vitamin A stimulates bone growth. In deficiency of ascorbic acid cartilage grows normally but fibroblasts fail to form osteoid. In deficiency of calcium or phosphorus or vitamin D, or in the presence of abnormal dietary ratios of calcium and phosphorus, there is defective deposition of these two elements in growing cartilage and osteoid.

The clinical diagnosis of active rickets is very difficult except in gross forms. At about 3 months of age, craniotabes is the most useful sign; at about 6 months, enlargement of the chosto-chondral junctions; towards the end of the first year, enlargement of the epiphyses at the wrist; and after the eighteenth month, delayed closure of the anterior fontanelle should be considered. The question of diagnosis of rickets is considered in the excellent review of Eliot and Park (1943) and in the report of the British Paediatric Association (1944); the latter shows the great variability in the clinical assessment of the incidence. The earliest diagnosis can be made by chemical methods; next by radiology; last by a clinical examination. By this examination alone it may be impossible to differentiate between rickets that is active or has been cured for months or even years. Radiological estimations of skeletal maturity and skeletal mineralization may prove valuable; they are discussed in the publications of Mack whose extensive studies will be very helpful (Mack and Smith, 1939).

Heart muscle in lower animals is affected by deficiencies of thiamine, potassium, and vitamin E; and deficiency of vitamin E affects skeletal muscle. But there is no clear evidence that vitamin E is necessary in man: there is no valid reason for believing it prevents abortion in women

or sterility in men or muscular dystrophies; and the latest paper by Vogelsang and colleagues (1947) wastes further paper and time. Muscle tenderness is a useful sign, since it appears relatively early in deficiency of thiamine. Winged scapulae and other postural defects are of very doubtful value.

Respiratory System. In deficiency of vitamin A, the ciliated columnar cells of the trachea and bronchi become replaced by keratinizing epithelium. This may block bronchi les, causing atelectasis. Dr. Jonxis of Rotterdam found that this occurred during the famine in the Netherlands, and a radiological examination of the chest was useful in revealing it.

Circulatory System. Heart muscle and changes in blood pressure and pulse rate have been mentioned elsewhere.

Nutritional edema may be divided into three types (Sinclair, 1948a). First, there is wet beriberi caused by deficiency of thiamine and a diet relatively high in carbohydrate. Secondly, there is hypoproteinemic edema caused by a lowered concentration of protein, particularly albumin, in the plasma with a consequent fall in osmotic pressure; but deficiency of aliments, such as occurred in concentration camps during the war, can lead to gross emaciation and very low values for plasma protein without edema. Thirdly, there is what may reasonably be called "famine edema" since it arises in persons who subsist on diets deficient in aliments; the persons are not necessarily emaciated and values for plasma protein are usually within the statistically normal range. The diagnosis of this third type in its mild forms is very difficult, since the person may appear to be well nourished: we have seen in Civil Internment Camps in Germany many males high in mesomorphy and low in gynandromorphy who at a glance appear well nourished and yet show pitting nutritional edema. The diagnosis must be made on the dietary history, medical history (especially nycturia) and other supporting signs (bradycardia, low blood pressure, acrocyanosis, etc.); and it is obvious that nonnutritional causes must be eliminated.

In erythropoiesis, various nutrients are probably concerned: certain essential amino acids, iron, copper, cobalt, pteroylglutamic acid, pyridoxine, riboflavin, and niacin. Deficiency of iron, copper, or pyridoxine causes microcytic hypochromic anemia. Pallor of the skin, nail bed, and mucous membranes is very deceptive in many cases; in deficiency of aliments there is a curious greyish-lemon tinge of the skin.

Gastrointestinal System. This is not normally examined in assessment of nutriture, although obviously abdominal signs will be sought if there is special indication. The very extensive work that has been done on damage to the liver by nutritional deficiency cannot be discussed here.

Genitourinary System. Testicular atrophy occurs in many nutritional deficiencies, and deficiency of arginine appears to cause decreased spermatogenesis in man (Holt *et al.*, 1942).

Amenorrhea can be caused by deficiency of aliments, but circumstances that accompany undernourishment may cause psychical amenorrhea: we noted during the famine in the Netherlands that some women who had complained of this sign started to menstruate again on or about the day of liberation.

VII. DISCUSSION—SUMMARY

The *nutriture* of the body depends upon the *nutrition* of the cells of the body, and this process involves *aliments* (which include substances that are used by the body for the production of energy, such as carbohydrate, fat and protein), *nutrients* (which include essential amino acids, essential fatty acids, vitamins, provitamins and compounds of certain essential elements), oxygen and water. Nutrition may also be affected by the accumulation of metabolic products or the presence of toxic substances, or by the action of physical agents such as heat or ultraviolet radiations. *Health* depends upon the degree of nutriture and upon the presence or absence of nonnutritional disease; the *physical state* of the body depends upon health and upon the presence or absence of structural defects which may or may not have been caused by past malnutriture. There are three important *properties of nutriture*: *quality* expresses the kinds or types of nutritional deficiencies; the *degree* of nutriture may arbitrarily be classified into five categories with death from surfeit or deficiency as limits; the *duration* of nutriture describes the time during which the state has existed. The acuteness or chronicity of the process causing the state is determined by the rate of change of degree with respect to time. The *development of malnutriture* usually occurs in four stages: inadequate ingestion, decreased bodily reserves, impaired function, anatomical lesions. Dietary, chemical, functional, and clinical methods may therefore be used; since growth and body weight are affected by nutriture, somatometric methods are also relevant. With any of these methods, the *concept of normality* is involved. Two definitions of normal, one based on physiological and the other on statistical concepts, are used: the condition of the body, or of some part or function of the body, is (physiologically) normal when it allows the usual functions to be adequately performed in the usual environment; a value of a variate is statistically normal when it does not differ from the mean by more than the standard deviation.

Dietary methods usually cannot alone be used to assess nutriture. *Chemical methods* are very valuable, and include estimations of substances

in tissues, blood, or urine. The composition of tissues alters before structural changes occur through malnutrition; estimations of vitamins of the B complex and of ascorbic acid in tissues such as leucocytes are particularly useful. Compounds in plasma vary in their behavior: some, such as carotenoids and ascorbic acid, tend to reflect the immediate past intake; others, such as niacin and riboflavin and usually protein, remain at concentrations that are within the statistically normal range even though clinical malnutrition may be present. In these cases a single estimation that falls within the statistically normal range gives little information. Estimations upon urine, with or without test doses, have not yet proved valuable for assessing nutrition.

The most useful of the *functional methods* is the measurement of the rod threshold of the dark-adapted eye provided a high value is tested again after therapy with vitamin A. The measurement of nerve accommodation may prove to be of similar value in detecting deficiency of thiamine. Measurements of the fragility of capillaries in the skin or gums are not helpful in assessing deficiency of ascorbic acid. Measurements of strength with dynamometers serve no useful purpose in nutritional surveys, and in these it is usually impracticable to include tests of physical fitness, of capacity for physical work, or of fatigue; a reliable test of endurance should be useful particularly in longitudinal studies.

Somatometric methods, such as the use of sex-age-height-weight tables or the calculation of various indices based on body measurements, are by themselves useless in the cross-sectional assessment of nutrition in an individual. A large number of nonnutritional factors condition growth, and malnutrition does not necessarily retard growth or diminish adult weight. Further, the optimum rate of growth of children is not necessarily the maximum. Even longitudinal studies of growth or of adult body weight are not simple. Measurement of the thickness of skin and subcutaneous tissue over the arm is useful in nutritional surveys.

Clinical methods are essential in the proper assessment of nutrition. The general assessment by inspection is usually concerned mainly with physical state, is strongly influenced by body size, and is found not to be reproducible. Symptoms, though subjective, should be recorded. The interpretation of clinical signs is difficult and very often erroneous; almost all the early signs are unspecific. Artifacts or the results of other non-nutritional factors may be misleading, such as unkempt hair, a smooth tongue caused by an upper denture, cracked lips or chapped skin caused by exposure, sun burn or snow blindness, bug-bites or "vagabond's disease." The differential diagnosis of advanced nutritional disease is usually relatively easy, although mistakes, as between pellagra and lupus erythematosus for example, may be made; but the appreciation of the

existence and cause of early departures from normality is difficult. Thorough familiarity with the normal is needed, and this is assisted by various aids to the examination such as the biomicroscope. The tissues that are most often affected structurally by malnutrition are the epidermis, and the cornea.

The *assessment of nutrition* is a diagnosis and, like clinical diagnosis, uses various disciplines. The combination of different methods, such as are mentioned above, is essential. These methods should, whenever possible, be quantitative and possess both precision and specificity so that the facts obtained are adequately described and are reproducible; they can be interpreted anew or reassessed as knowledge advances. The facts should be objective; the interpretation of the facts is subjective. Measurements, like those made by chemical methods, are therefore particularly valuable. Clinical methods are unfortunately subjective to a large degree, and the criteria adopted by the examiner inevitably alter: the standard of normality tends to be affected by what is commonly found in the particular population under examination, and even by what the examiner expects to find. The assessment of deviations from the normal demands a knowledge of the range of normality, and of the factors that enlarge or diminish it. Unfortunately, the study of the relevant parts of the physiology, morphology and chemistry of man has not advanced far; man is a neglected animal and the variations that are found and the causes of these should be more carefully studied. It is obvious that if a nutritional variate is measured in two populations of different nutritures, one normal and one poor, and if the distribution of the variate in each is defined by an approximately Gaussian curve, the two curves are likely to overlap considerably. A significant difference between the means of the values of the variate in the two populations may be found, and this is important in comparing the nutritures of the two populations. But since the values of the variate in the individuals overlap, a single observation in one individual may not give much relevant information: the value may be in the upper range of the deficient group or in the lower range of the normal group. Since a variate has a probability function, our statistical definition of normality would most satisfactorily be in terms of probability; but for practical convenience a less satisfactory definition in terms of the standard deviation has been adopted, although the distribution of the values of the variate may not be approximately Gaussian. Such unsatisfactory practices must be adopted until we know more about the causes of variation and the values of variates at which abnormality, in terms of the physiological definition of normal, arises. For instance, let us assume that vitamin A is measured in the serum of a large number of apyrexial adults all of whom have nor-

mal nutriture regarding this vitamin, and that the mean is 100 arbitrary units and the standard deviation 33. Then, according to our definition of statistical abnormality, individuals having values below 67 or above 133 units would be abnormal. This of course does not mean that they have malnutriture regarding vitamin A: we have already said that they have normal nutriture regarding this vitamin. Similarly we might say that the values falling below 34 or above 166 units (that is, outside the limits of twice the standard deviation from the mean) are extremely abnormal. If the distribution of the values is Gaussian, about 84% of the values will not be abnormally low, about 13½% of the values will be abnormally low but not extremely abnormal, and about 2¼% of the values will be in the lowest category of abnormality. If however it were found that a value below, say, 30 units maintained for a long time was invariably accompanied by a significantly increased rod threshold of the dark-adapted eye whereas a value not less than 30 units was similarly not accompanied by a significant increase in rod threshold, and if a significant increase in rod threshold were the first indication of poor nutriture regarding vitamin A, then we would be justified in taking a value below 30 units as indicating abnormality in the physiological sense. In practice we have not the requisite information to analyse variates, such as chemical levels in blood or dietary requirements of aliments and nutrients, in terms of either the physiological or the statistical definition of normality. Despite this, arbitrary limits must be adopted as a practical convenience. Those used, for chemical and for two functional estimations, by the Oxford Nutrition Survey early in its work are listed in Table I (Sinclair, 1947a, corrected). Data have now been collected that will shortly make it possible to amend these limits and make them less arbitrary.

Nutritionists, school medical officers and others concerned with the public health often try to assess general nutriture in individuals. The attempt smacks of the alchemist's search for the philosopher's stone. Spearman in his studies of intelligence showed that there was a general factor (g) and specific factors (s); it is sometimes thought that general nutriture (which we may call N) can similarly be rated in individuals. The general nutriture of the body is the sum of the specific nutritures (N_s) of the cells of the body, N_s depending upon the factors (such as nutrients) already discussed as affecting the nutrition of cells. N is a statistical concept and can be assessed only in terms of its effect on health; this can only be measured quantitatively in terms of probability of death (which is zero health). Whereas we cannot therefore measure N , or for example say whether a child with severe active rickets has worse general nutriture than one with mild xerophthalmia and mild scurvy, except in terms of probability of death occurring if the state is continued,

TABLE I

Arbitrary Standards of Normality Used by the Oxford Nutrition Survey

	Estimated on	Units/100 ml. or 100 g.	Abnormal below	Extreme abnormality below
Hemoglobin:				
<15 years, male	B	g.	13.85	12.05
<15 years, female	B	g.	12.50	10.70
7-14 years	B	g.	12.05	10.25
2-6 years	B	g.	11.15	9.35
0-1 years	B	g.	10.25	8.45
Erythrocrit:				
<15 years, male	B	%	41	36
<15 years, female	B	%	37	32
7-14 years	B	%	36	30
2-6 years	B	%	33	28
0-1 years	B	%	30	25
Leucocrit, low	B	%	0.5	0.3
high	B	%	1.0 ¹	1.5 ¹
Protein, total	S	g.	6.1	5.3
Albumin	S	g.	4.0	3.2
Vitamin A (adults)	S	I.U.	70	30
Carotenoids	S	γ	100	50
Phosphatase (adults)	S	K.-A. units	10 ¹	16 ¹
Thiamine hydrochloride	B	γ	4.5	2.0
Pyruvic acid	B	mg.	1.6 ¹	2.0 ¹
Niacin	B	γ	400	200
Riboflavin	B	γ	20	12
	L	γ	400	200
Ascorbic acid	B	mg.	0.50	0.10
	L	mg.	20	10
Fasting urine (per hour):				
Thiamine hydrochloride	U	γ	7.50	2.51
N'-Methylnicotinamide	U	γ	95.0	45.1
Riboflavin	U	γ	30.0	10.1
Ascorbic acid	U	mg.	0.46	0.15
Rod threshold	Dark-adapted eye	log. μmL.	2.1 ¹	2.5 ¹
Capillary fragility	Skin of fore- arm	Petechiae per sq. in.	10 ¹	20 ¹

B = Whole blood

S = Serum

L = Leucocyte-platelet layer

U = Urine

¹ Abnormal and extremely abnormal values *not less than* these limits.

we can attempt to assess specific nutriture regarding aliments or nutrients by the methods discussed in this article. But *N* is not merely the sum of the specific nutritures that we can assess: just as the experienced clinician can tell that a child is very ill without the aid of a cachometer, so the experienced nutritional observer on the basis of his physical examination can assess the person's nutriture partly by taking into account the presence and combination of the signs discussed above and partly by an ill-defined belief that the person has a particular degree of nutriture. This assessment by the physical examination alone differs from the rapid general assessment by inspection because the former is based on the examination of particular systems (skin, eyes, etc.) whereas the latter is an assessment mainly of physical state. A rapid nutritional survey should include a medical history and a clinical examination with the symptoms and signs being recorded in three grades of abnormality, "mild," "marked," or "severe"; then a tentative clinical diagnosis and a provisional assessment of general nutriture should be made. On the basis of these and of the results of chemical, functional and dietary methods the final diagnosis and assessment of *N* are made. It must be clearly understood that this assessment of *N* is relative only: the more detailed and extensive the methods employed in the assessment, the lower will this be rated. If, for example, the methods we happened to be using gave no information about mild deficiency of ascorbic acid, we should tend to class the individuals as having normal nutriture regarding this nutrient; but if estimations of the nutrient in blood or plasma or leucocytes are included, the assessed state cannot be improved and will probably be lowered. Therefore if comparisons between the general nutritures of individuals or of populations are being made, the methods included must be the same. These methods, which have been discussed in this article, are summarized in Table II.

It is evident therefore that the assessment of nutriture in populations is not, as some seem to think, a matter of training a girl for a couple of days before sending her into the wilds with a box of forms and a slit-lamp microscope; it is a detailed undertaking for a team of workers experienced in different disciplines. The cross-sectional assessment in a single individual is the hardest task, the comparison of two populations by cross-sectional assessments being less difficult. Longitudinal studies are much simpler, whether they follow the nutritures of single individuals or of populations; and if controlled therapeutic tests can be included in either of these, the most satisfactory position is reached. The problem however still remains complex: an increase in the amounts of ascorbic acid in the bloods of the group treated with this nutrient may imply no alteration in the nutriture of the individuals in the group.

TABLE II

Methods for Use in Rapid Assessments of Nutriture in Populations

Primary. A clinical examination, a dietary history and a medical history are *essential*. The other methods placed in the Primary group are *valuable*.

Secondary. The methods in this category are either (a) valuable but need special apparatus or techniques, or (b) simple to perform but need further research for their interpretation or are otherwise of doubtful value.

Tertiary. The methods in this category have no proved value as yet for assessing nutriture in cross-sectional studies.

B indicates determinations on whole blood, L on leucocytes, S on serum or plasma.

Primary	Secondary	Tertiary
<i>Dietary</i>		
Dietary history Family inventory and purchase method.	(a) Individual method	
<i>Chemical</i>		
Hemoglobin (B)	(a) Pyruvate (B)	Minerals (B, S)
Erythrocrit (or erythrocyte count) (B)	(a) Protein with fractionation (S)	Prothrombase clotting-time (B)
Protein without fractionation (S)	(a) Ascorbic acid (L)	Vitamins of the B complex (B)
Ascorbic acid (B)	(a) Vitamins of the B complex (L)	Saturation-tests of water-soluble vitamins
Vitamin A and carotenoids (S)	(b) Phosphatase in adults (S)	
Phosphatase in children (S)	(b) Fasting urinary excretion tests or load tests of water-soluble vitamins	
<i>Functional</i>		
	(a) Rod threshold of dark-adapted eye (b) Endurance tests	Visual acuity Nerve accommodation Ergometric tests Psychometric tests Electrocardiograms
<i>Somatometric</i>		
Weight Height Thickness of skin and subcutaneous tissue	(b) Sitting-height	Other somatometric determinations
<i>Clinical</i>		
Examination of skin, eyes, etc. Medical history Blood pressure	(b) Pulse-rate (a) X-ray of hand and wrist in children	X-ray of hand and wrist in adults Capillary fragility Gum fragility

There is no easy method of evaluating *N*, although relevant observations and estimations of deceitfully enticing simplicity may be made. If we may allow ourselves the luxury of a metaphor, we may conclude that through the wilderness of human nutrition there run many paths, some twisted, many beset with snares, and all thorny; the only one leading straight forward is the path of scientific method which is laid by the critical accumulation and sifting of orderly truths, and this path is largely untrodden.

In this article I have drawn upon experience gained by the Oxford Nutrition Survey. I am particularly indebted to two former members of its staff, Mr. B. B. Lloyd and Mr. M. C. K. Tweedie, for their helpful suggestions.

REFERENCES

- Abelin, I., and Rhyn, E. 1942. *Z. Vitaminforsch.* **12**, 56.
- Adamson, J. D., Jolliffe, N., Kruse, H. D., Lowry, O. H., Moore, P. E., Platt, B. S., Sebrell, W. H., Tice, J. W., Tisdall, F. F., Wilder, R. M., and Zamecnik, P. C. 1945. *Can. Med. Assoc. J.* **52**, 227.
- Adcock, E. W., Hammond, W. H., and Magee, H. E. 1947. *J. Hyg.* **45**, 65.
- Adolph, W. H., Greaves, A. V., Lawney, J. C., and Robinson, H. L. 1944. *War Med.* **5**, 349.
- Albanese, A. A., Randall, R. M., and Holt, L. E., Jr. 1943. *Science* **97**, 312.
- Ansbacher, S. 1941. *Science* **93**, 164.
- Archdeacon, J. W., and Murlin, J. R. 1944. *J. Nutrition* **28**, 241.
- Bacharach, A. L., Coates, M. E., and Middleton, T. R. 1942. *Biochem. J.* **36**, 407.
- Baird, D. 1945. *J. Obstetr. Gynaecol. Brit. Empire* **52**, 217, 339.
- Balfour, M. I. 1938. *Proc. Roy. Soc. Med.* **31**, 911-914.
- Balfour, M. I. 1944. *Lancet* **i**, 208.
- Barborka, C. J., Foltz, E. E., and Ivy, A. C. 1943. *J. Am. Med. Assoc.* **122**, 717.
- Benesch, R. 1945. *Lancet* **i**, 718.
- Bessey, O. A., Lowry, O. H., and Brock, M. J. 1947. *J. Biol. Chem.* **168**, 197-205.
- Board of Education, London. 1934. Admin. Mem. No. 124.
- Booher, L. E., Callison, E. C., and Hewston, E. M. 1939. *J. Nutrition* **17**, 317-331.
- Borsook, H., Dubnoff, J. W., Keighley, G., and Wiehl, D. G. 1946. *Milbank Mem. Fund Quart.* **24**, 99.
- Bransby, E. R., Hunter, J. W., Magee, H. E., Milligan, E. H. M., and Rodgers, T. S. 1944. *Brit. Med. J.* **i**, 77.
- Brenner, S., and Roberts, L. J. 1943. *Arch. Internal Med.* **71**, 474.
- British Paediatric Association. 1944. HMSO, London Ministry of Health: Reports on Public Health and Medical Subjects No. 92.
- Browne, J. A. 1939. *Caribbean Med. J.* **1**, 218.
- Brozek, J., Guetzkow, H., Mickelsen, O., and Keys, A. 1946. *J. Applied Psychol.* **30**, 359.
- Buschke, W. 1943. *Arch. Ophthalmol. Chicago* **30**, 735.
- Butler, A. M., and Cushman, M. 1940. *J. Clin. Invest.* **19**, 459.
- Calhoun, F. P. 1918. *Am. J. Ophthalmol.* **1**, 834; *Ophthalmic Record* **26**, 63.
- Cayer, D., Ruffin, J. M., and Perlzweig, W. A. 1945. *Southern Med. J.* **38**, 111.
- Clark, W. E. le G. and Buxton, L. H. D. 1938. *Brit. J. Dermatol. Syphilis* **50**, 221.

- Cogswell, R. C., Berryman, G. H., Henderson, C. R., Denko, C. W., and Spinella, J. R. 1946. *Am. J. Physiol.* **147**, 39.
- Crandon, J. H., Lund, C. C., and Dill, D. B. 1940. *New Engl. J. Med.* **223**, 353-369.
- Csik, L., and Bencsik, J. 1927. *Klin. Wochschr.* **6**, 2275.
- Dann, W. J., and Darby, W. J. 1945. *Physiol. Revs.* **25**, 326.
- Day, P. L., Darby, W. J., and Cosgrove, K. W. 1938. *J. Nutrition* **15**, 83.
- Deggeller, C., Jr. 1936. *Acta Brevia Neerland Physiol. Pharmacol. Microbiol.* **6**, 1.
- Denny-Brown, D. 1947. *Medicine* **26**, 41.
- Drigalski, W. von. 1939. *Z. Vitaminforsch.* **9**, 325.
- Droese, W. 1941. *Münch. med. Wochschr.* **88**, 909.
- Ebbs, J. H., Brown, A., Tisdall, F. F., Moyle, W. J., and Bell, M. 1942a. *Can. Med. Assoc. J.* **46**, 6.
- Ebbs, J. H., Scott, W. A., Tisdall, F. F., Moyle, W. J., and Bell, M. 1942b. *Can. Med. Assoc. J.* **46**, 1.
- Ebbs, J. H., Tisdall, F. F., and Scott, W. A. 1941. *J. Nutrition* **22**, 515.
- Egaña, E., Johnson, R. E., Bloomfield, R., Brouha, L., Meiklejohn, A. P., Whittenberger, J., Darling, R. C., Heath, C., Graybiel, A., and Consolazio, F. C. 1942. *Am. J. Physiol.* **137**, 731.
- Eliot, M. M., and Park, E. A. 1943. *Brennemann's Practice Pediat.* **1**, Chap. 36.
- Elsom, K. O., Lewy, F. H., and Heublein, G. W. 1940. *Am. J. Med. Sci.* **200**, 757.
- Follis, R. H., Jr. 1942. *Bull. Johns Hopkins Hosp.* **71**, 235.
- Follis, R. H., Jr., Day, H. G., and McCollum, E. V. 1941. *J. Nutrition* **22**, 223.
- Foltz, E. E., Ivy, A. C., and Barborka, C. J. 1942. *J. Lab. Clin. Med.* **27**, 1396.
- Franklin, K. J. 1938. *Lancet* **ii**, 1287.
- Frantzell, A., Törnquist, R., and Waldenström, J. 1945. *Acta Med. Scand.* **122**, 207.
- Franzen, R. 1929. "Physical Measures of Growth and Nutrition." American Child Health Association, New York.
- Gastpar. 1908. *Z. Schulgesundheits Pfl.* **21**, 689.
- Göthlin, G. F. 1931. *Skand. Arch. Physiol.* **61**, 225.
- Gounelle, H. 1940. *Bull. mém. soc. méd. hôp. Paris* **56**, 255.
- Grande, F., and Peraita, M. 1941. Avitaminosis y Sistema Nervoso. Editorial "Miguel Servet," Madrid-Barcelona.
- Graves, B. 1934. *Brit. J. Ophthalmol.* **18**, 305.
- Harrell, R. F. 1943. Effect of Added Thiamine on Learning. Columbia University Press, New York.
- Harrell, R. F. 1946. *Teachers Coll. Rec.* **47**, 257.
- Health, Ministry of. 1937. First Report of the Advisory Committee on Nutrition. HMSO, London.
- Hecht, A. F. 1907. *Jahrb. Kinderheilk.* **65**, 113.
- Hecht, S., and Mandelbaum, J. 1940. *Am. J. Physiol.* **130**, 651.
- Hogarth, A. H. 1909. Medical Inspection of Schools. Oxford University Press, London.
- Holt, L. E., Jr., Albanese, A. A., Shettles, L. B., Kajdi, C., and Wangerin, D. M. 1942. *Federation Proc.* **1**, 116.
- Isaacs, B. L., Jung, F. T., and Ivy, A. C. 1940. *Arch. Ophthalmol.* **24**, 698.
- Ivy, A. C. 1944. *Quart. Bull. Northwestern Univ. Med. School* **18**, 22.
- Johnson, R. E., Darling, R. C., Forbes, W. H., Brouha, L., Egaña, E., and Graybiel, A. 1942. *J. Nutrition* **24**, 585.
- Johnson, R. E., Henderson, C., Robinson, P. F., and Consolazio, F. C. 1945. *J. Nutrition* **30**, 89.

- Jolliffe, N., and Most, R. M. 1943. *Vitamins and Hormones* **1**, 59.
- Jones, R. H. 1938. *J. Roy Statistical Soc. London* **101**, 1.
- Kagawa, S. 1938. *Japan. J. Med. Sci.* VIII, *Internal Med. Pediat. Psychiat.* **5**, 1.
- Kajdi, L., Light, J., and Kajdi, C. 1939. *J. Pediat.* **15**, 197.
- Keys, A. 1943a. *Federation Proc.* **2**, 164.
- Keys, A. 1943b. *Bull. Minnesota Med. Foundation* **3-7**.
- Keys, A. 1946. *Occupational Med.* **2**, 536.
- Keys, A. 1947. *Nutrition Revs.* **5**, 129.
- Keys, A., and Henschel, A. F. 1942. *J. Nutrition* **23**, 259.
- Keys, A., Henschel, A. F., Mickelsen, O., Brozek, J. M., and Crawford, J. H. 1944. *J. Nutrition* **27**, 165.
- Keys, A., Henschel, A. F., Taylor, H. L., Mickelsen, O., and Brozek, J. M. 1945. *Am. J. Physiol.* **144**, 5.
- King, J. D. 1943. *Brit. Dental J.* **74**, 113 *et seq.*
- Knutson, J. W., and Armstrong, W. D. 1945. *U.S. Pub. Health Repts.* **60**, 1085.
- Kodicek, E., Carpenter, K. J., and Harris, L. J. 1946. *Lancet* **ii**, 491.
- Koppe, O. 1905. *Der Schularzt* **3**, 219.
- Kornfeld, W. and Schüller, H. 1930. *Z. Kinderheilk.* **49**, 277-298.
- Kruse, H. D. 1941. *Milbank Mem. Fund Quart.* **19**, 207.
- Kruse, H. D. 1942a. *Milbank Mem. Fund Quart.* **20**, 245.
- Kruse, H. D. 1942b. *Milbank Mem. Fund Quart.* **20**, 262.
- Kruse, H. D. 1942c. *Milbank Mem. Fund Quart.* **20**, 290.
- Kruse, H. D. 1943. "Medical Evaluation of Nutritional Status" in *Handbook of Nutrition*. American Medical Association, Chicago, Chap. XXII, pp. 425-471.
- Kruse, H. D., Bessey, O. A., Jolliffe, N., McLester, J. S., Tisdall, F. F., Wilder, R. M., and Sydenstricker, V. P. W. 1944. *Arch. Internal Med.* **74**, 258.
- Kruse, H. D., Sydenstricker, V. P., Sebrell, W. H., and Cleckley, H. M. 1940. *U.S. Pub. Health Repts.* **55**, 157.
- Landor, J. V., and Pallister, R. A. 1935. *Trans. Roy Soc. Trop. Med. Hyg.* **29**, 121.
- Lange, H. F. 1946. *Acta Med. Scand. Suppl.* CLXXVI, pp. 1-202.
- League of Nations. 1933. *Quart. Bull. Health Organisation League Nations* **2**, 116.
- Lecoq, R., Chauchard, P., and Mazoué, H. 1946. *Rev. path. comp. hyg. gén.* **46**, 531.
- Lewis, T. 1929. *Heart* **15**, 1.
- Lewis, T., Pickering, G. W., and Rothschild, P. 1929-31. *Heart* **15**, 359.
- Liebmann, J., Wortis, H., and Wortis, E. 1938. *Am. J. Med. Sci.* **196**, 388.
- Livingston, P. C. 1944. *Am. J. Ophthalmology* **27**, 349.
- Lowry, O. H., Bessey, O. A., Brock, M. J., and Lopez, J. A. 1946. *J. Biol. Chem.* **166**, 111.
- McCance, R. A., Widdowson, E. M., and Verdoe-Roe, C. M. 1938. *J. Hyg.* **38**, 596.
- McClendon, J. F. 1944. *Federation Proc.* **3**, 94.
- McClendon, J. F., and Foster, W. C. 1945. *Federation Proc.* **4**, 159.
- McCormick, W. J. 1940. *Med. Record* **152**, 439.
- Machella, T. E. 1942. *Am. J. Med. Sci.* **203**, 114.
- Mack, P. B., and Smith, J. M. 1939. *Penn. State Coll. Bull.* **33**, No. 43.
- Mackenzie, A. 1913. 7th Annual Report, Dunfermline 1912-13, Edinburgh.
- Magee, H. E. 1944. *Mon. Bull. Ministry of Health & Emergency Public Health Laboratory Service (MRC)* **3**, 146.
- Medical Research Council, AFFC, Vitamin A Sub-Committee. 1945. *Nature* **156**, 11.
- Melnick, D., Hochberg, M., and Oser, B. L. 1945. *J. Nutrition* **30**, 81.

- Métivier, V. M. 1941. *Am. J. Ophthalmol.* **24**, 1265.
- Meyer, E., and Meyer, M. B. 1944. *Bull. Johns Hopkins Hosp.* **74**, 98.
- Mickelsen, O. 1943. *J. Lancet* **63**, 360.
- Mickelsen, O., Caster, W. O., and Keys, A. 1947. *J. Biol. Chem.* **168**, 415.
- Milam, D. F. 1942. *Am. J. Pub. Health* **32**, 406.
- Milam, D. F., and Darby, W. J. 1945. *Southern Med. J.* **38**, 117.
- Miller, O. N., and Beach, E. F. 1945. Report on the Chemical Determination of Nutritional State of Full Duty Troops, Manila Area, July & August 1945.
- Milligan, E. H. M., and Lewis-Faning, E. 1942. *Medical Officer London* **67**, 85.
- Mitchell, H. H. 1932. *Human Biol.* **4**, 469.
- Mitchell, H. H. 1944. *J. Am. Dietet. Assoc.* **20**, 511.
- Mitra, K. 1943. *Indian Med. Gaz.* **78**, 330.
- Moore, D. F. 1939. *J. Trop. Med. Hyg.* **42**, 109.
- Moore, D. F. 1940. *Brit. Med. J.* **ii**, 848.
- Morell, T. 1940. *Deut. Med. Wochschr.* **66**, 398.
- National Research Council, Food & Nutrition Board. 1945. Recommended Dietary Allowances (Revised). Reprint & Circular Series No. 122.
- Nicholls, L. 1935. *Indian Med. Gaz.* **70**, 550.
- Oeder, G. 1910. *Med. Klinik.* **6**, 657.
- Orr, J. B. 1936. Food Health and Income. 2nd ed. Macmillan, London.
- Pallister, R. A. 1940. *J. Malaya Branch Brit. Med. Assoc.* **4**, 191.
- Patterson, J. M., and McHenry, E. W. 1941. *Can. Pub. Health J.* **32**, 362.
- People's League of Health. 1942. *Lancet* **ii**, 10.
- Perlzweig, F. A., Rosen, F., Levitas, N., and Robinson, J. 1947. *J. Biol. Chem.* **167**, 515.
- Pett, L. B. 1939. *J. Lab. Clin. Med.* **25**, 149.
- Pett, L. B. 1945. *Can. Pub. Health J.* **36**, 69.
- Pillat, A. 1931. *Z. Augenheilk.* **73**, 244.
- Prados, M., and Swank, R. L. 1942. *Arch. Neurol. Psychiat.* **47**, 626.
- Rapaport, H. G., Miller, S. H., and Sicular, A. 1940. *J. Pediat.* **16**, 624.
- Rich, W. M. 1946. *Brit. Med. J.* **i**, 330.
- Rosenbaum, E. E., Portis, S., and Soskin, S. 1942. *J. Lab. Clin. Med.* **27**, 763.
- Ruffin, J. M., and Cayer, D. 1944. *J. Am. Med. Assoc.* **126**, 823.
- Ryle, J. A. 1947. *Lancet* **i**, 1.
- Sandstead, H. R. 1943. *U.S. Pub. Health Repts., Suppl. No.* **169**.
- Scarborough, H. 1945. *Biochem. J.* **39**, 271.
- Scarborough, H., and Gilchrist, E. 1944. *Biochem. J.* **38**, i.
- Scott, H. H. 1918. *Ann. Trop. Med. Parasitol* **12**, 109.
- Sebrell, W. H., and Butler, R. E. 1938. *U.S. Pub. Health Repts.* **53**, 2282.
- Sebrell, W. H., and Butler, R. E. 1939. *U.S. Pub. Health Repts.* **54**, 2121.
- Semmons, E. M., and McHenry, E. W. 1944. *Can. Pub. Health J.* **35**, 286.
- Sinclair, H. M. 1942. *Chem. Prod.* **5**, 47.
- Sinclair, H. M. 1944. *Am. J. Pub. Health* **34**, 828.
- Sinclair, H. M. 1945. *Practitioner* **154**, 371.
- Sinclair, H. M. 1947a. "Biochemical Aids in the Diagnosis of Nutritional Deficiencies" in Recent Advances in Clinical Pathology. Ch. XVIII. J. & A. Churchill, London.
- Sinclair, H. M. 1947b. Communication to the XVII International Physiological Congress, July 1947. To be published.
- Sinclair, H. M. 1947c. *Abstr. World Med.* **2**, 360.

- Sinclair, H. M. 1948a. *Proc. Roy. Soc. Med.* **41**, 541.
- Sinclair, H. M. 1948b. *Brit. J. Nutrition* **2**, 161.
- Smith, C. A. 1947a. *J. Pediat.* **30**, 229.
- Smith, C. A. 1947b. *Am. J. Obstet. Gynecol.* **53**, 599.
- Spillane, J. D. 1945. *Lancet* ii, 317.
- Spillane, J. D. 1947. *Nutritional Disorders of the Nervous System*. E. & S. Livingstone Ltd, Edinburgh.
- Stannus, H. S. 1944. *Brit. Med. J.* ii, 103.
- Steffens, L. F., Blair, H. L., and Sheard, C. 1940. *Am. J. Ophthalmol.* **23**, 1325.
- Steven, D. M. 1942. *Trans. Ophthalmol. Soc. U.K.* **62**, 259.
- Strachan, H. 1888. *Ann. Universal Med. Sci.* **1**, 139.
- Sullivan, M., and Evans, V. J. 1944. *J. Nutrition* **27**, 123-139.
- Sullivan, M., and Evans, V. J. 1945. *Arch. Dermatol. and Syphilol.* **51**, 17.
- Sullivan, M., and Nicholls, J. 1940. *J. Investigative Dermatol.* **3**, 317.
- Sullivan, M., and Nicholls, J. 1941. *J. Investigative Dermatol.* **4**, 181.
- Sullivan, M., and Nicholls, J. 1942a. *Arch. Dermatol. and Syphilol.* **45**, 917.
- Sullivan, M., and Nicholls, J. 1942b. *Arch. Dermatol. and Syphilol.* **45**, 295.
- Sure, B., and Dichek, M. 1941. *J. Nutrition* **21**, 445.
- Sutherland, I. 1946. *Lancet* ii, 953.
- Swank, R. L. 1940. *J. Exptl. Med.* **71**, 683.
- Swank, R. L., and Bessey, O. A. 1941. *J. Nutrition* **22**, 77.
- Sydenstricker, V. P. 1941. *Am. J. Pub. Health* **31**, 344.
- Sydenstricker, V. P., Hall, W. K., Hock, C. W., and Briggs, A. P. 1946. *Federation Proc.* **5**, 242.
- Sydenstricker, V. P., Sebrell, W. H., Cleckley, H. M., and Kruse, H. D. 1940. *J. Am. Med. Assoc.* **114**, 2437.
- Sydenstricker, V. P., Singal, S. A., Briggs, A. P., DeVaughan, N. M., and Isbell, H. 1942. *J. Am. Med. Assoc.* **118**, 1199.
- Taylor, H. L., Brozek, J., Henschel, A. F., Mickelsen, O., and Keys, A. 1945. *Am. J. Physiol.* **143**, 148.
- Tisdall, F. F., McCreary, J. F., and Pearce, H. 1943. *Can. Med. Assoc. J.* **49**, 5.
- Totter, J. R., and Day, P. L. 1942. *J. Nutrition* **24**, 159.
- Tuxford, A. W. 1917. *Sch. Hyg.* **8**, 65.
- Vail, D., and Ascher, K. W. 1942. *Trans. Am. Ophthalmol. Soc.* **40**, 181-214.
- Victor, J. 1934. *Am. J. Physiol.* **108**, 229.
- Vogelsang, A. B., Shute, E. V., and Shute, W. E. 1947. *Med. Record* **180**, 21.
- Wagner, K. H. 1940. *Z. physiol. Chem.* **264**, 153.
- Wald, G. 1941. *J. Optical Soc. Am.* **31**, 235.
- Wald, G., Brouha, L., and Johnson, R. E. 1942. *Am. J. Physiol.* **137**, 551.
- Wald, G., Jeghers, H., and Arminio, J. 1938. *Am. J. Physiol.* **123**, 732.
- Wald, G., and Steven, D. M. 1939. *Proc. Nat. Acad. Sci. U.S.* **25**, 344.
- Wetzel, N. C. 1941. *J. Am. Med. Assoc.* **116**, 1187.
- Wetzel, N. C. 1943. *J. Pediat.* **22**, 82, 208, 329.
- White, C. 1934. *J. Am. Med. Assoc.* **102**, 2178-2179.
- Widdowson, E. M. 1936. *J. Hyg.* **36**, 269.
- Widdowson, E. M. 1947. *Medical Research Council Spec. Rep. Series No. 257*.
- Widdowson, E. M., and Alington, B. K. 1941. *Lancet* ii, 361.
- Widdowson, E. M., and McCance, R. A. 1936. *J. Hyg.* **36**, 293.
- Widdowson, E. M., and McCance, R. A. 1942. *Lancet* ii, 689.
- Widdowson, E. M., and McCance, R. A. 1943. *Lancet* i, 230.

- Wilkinson, P. B. 1944. *Lancet* ii, 655.
- Wilkinson, P. B., and King, A. 1944. *Lancet* i, 528.
- Williams, J. Rhys. 1937a. *Public Health, London*, **50**, 231.
- Williams, J. Rhys. 1937b. *Public Health, London* **51**, 59.
- Williams, J. Rhys. 1938. *Public Health, London* **51**, 185.
- Williams, J. Rhys. 1939. *Public Health, London* **52**, 314.
- Williams, R. D., Mason, H. L., Power, M. H., and Wilder, R. M. 1943. *Arch. Internal Med.* **71**, 38.
- Williamson, R. 1941. *Biochem. J.* **35**, 1003.
- Wolbach, S. B. 1945. *Nutrition Revs.* **3**, 193.
- Woolley, D. W. 1941. *J. Biol. Chem.* **139**, 29.
- Woolley, D. W. 1946. *J. Biol. Chem.* **163**, 773.
- Wright, E. J. 1928. *W. African Med. J.* **2**, 127.
- Youmans, J. B., and Patton, E. W. 1942. *Clinics* **1**, 303.
- Young, C. M., and McHenry, E. W. 1942. *Can. Pub. Health J.* **33**, 224.
- Young, C. M., and McHenry, E. W. 1943. *Can. Pub. Health J.* **34**, 367.
- Ziskin, D. E. 1937. *J. Dental Research* **16**, 367.

Vitamins in Microorganisms—Distribution and Quantitative Synthesis

By J. M. VAN LANEN AND FRED W. TANNER, JR.¹

*Hiram Walker and Sons, Inc. and Northern Regional Research Laboratory, U. S.
Department of Agriculture, Peoria, Illinois*

CONTENTS

	<i>Page</i>
I. Introduction.....	164
II. Thiamine.....	166
1. Content of Microorganisms.....	166
2. Microbial Synthesis.....	167
3. Synthesis from Precursors.....	170
4. Absorption by Microorganisms.....	171
5. Thiamine Inactivation.....	173
III. Riboflavin.....	173
1. Synthesis by Bacteria.....	174
2. Synthesis by Yeasts.....	178
3. Synthesis by Molds.....	180
IV. Pantothenic Acid.....	181
1. Synthesis by Microorganisms.....	181
2. Synthesis from Precursors.....	183
V. Niacin.....	183
1. Content of Microorganisms.....	183
2. Synthesis of Niacin.....	185
3. Adsorption from the Medium.....	187
4. Niacin Inactivation.....	187
VI. Biotin.....	188
1. Biotin in Microorganisms.....	189
2. Absorption by Microbial Cells.....	190
3. Biotin Analogues.....	190
VII. <i>p</i> -Aminobenzoic Acid.....	191
1. Production by Microorganisms.....	191
2. Factors Influencing Synthesis.....	193
VIII. Pyridoxine.....	194
IX. Inositol.....	196
X. Folic Acid.....	197
1. Content of Microorganisms.....	198
2. Biosynthesis of Folic Acid.....	200
3. Production from Precursors.....	201
XI. Ascorbic Acid.....	202

¹ Present address: Chas. Pfizer and Co., Inc., Brooklyn, N. Y.

	<i>Page</i>
XII. Ergosterol.....	204
1. Ergosterol in Bacteria.....	204
2. Ergosterol in Molds.....	204
3. Synthesis by Yeasts.....	206
XIII. Vitamin K.....	207
XIV. Carotenoids.....	208
XV. Vitamins in Fermentation Products.....	210
XVI. Summary.....	212
References.....	214

I. INTRODUCTION

Within the past decade our knowledge regarding the existence of new nutritional factors and the manner in which these and earlier recognized vitamins function in metabolism has been greatly extended. Conspicuous as a result of this progress has been the growing realization that these substances have a general rather than a particular significance in biology. Consequently, nutrition studies irrespective of whether they are concerned with higher plants, animals, or fungi have been integrated and share common ground and common objectives. Particularly striking, for example, has been the parallelism between factors essential in animal nutrition and those required for the growth of microorganisms. This similarity has provided an excellent means of investigating these substances since microorganisms have well-defined and often simple requirements and are especially suitable for the examination of isolated biochemical reactions. Actually, the remarkable success which has attended their application has made microorganisms the tool of choice for elucidating the role of vitamins in metabolism. Based on contributions to our knowledge of the structure and function of biotin, pyridoxine, pantothenic acid, folic acid, and *p*-aminobenzoic acid as well as other nutrilites, microbiology is certain to occupy a pivotal position in furthering our understanding of essential nutritional factors.

The most commonly applied and productive approaches to nutritional problems employing lower fungi have involved one of the following: (a) determination of the growth requirements of various species and their derived mutants, (b) determination of the coenzyme requirements of certain enzymes, (c) metabolite-antimetabolite relationships, and (d) the ability of certain species to utilize vitamin precursors and vitamin analogues. Another approach which should provide at least supplemental information relative to the mechanism of vitamin action is that offered by the study of quantitative biosynthesis of vitamins under a wide variety of experimental conditions. Such studies have been initiated only recently and represent a natural progression from earlier

qualitative investigations in which synthesis was assumed when growth occurred in the absence of an external supply of the factor under study. The examination of synthetic abilities, while not developed systematically, has been extended to numerous species. The influence of certain physical and chemical factors upon the degree of activity has been ascertained, including interesting observations linking inorganic elements with vitamin formation. Evidence also has accumulated to show that vitamins not only are synthesized but are absorbed, excreted, and destroyed by microorganisms, the rate and extent of these activities varying with the species and cultural conditions. Needless to say, much further work will be necessary to determine whether such behavior is relevant to either the requirements or the specific functions of these factors for various species.

Aside from theoretical considerations, the elaboration of vitamins by microorganisms has become of increasing commercial importance. Certain species capable of producing extraordinary amounts of riboflavin, pantothenic acid, biotin, and folic acid have been discovered and others have been described which acquire and store substantial quantities of thiamine and niacin. Some of these have been put to industrial use and others appear to be worthy of consideration in this respect. Other than microbiological processes designed specifically for vitamin production, the biosynthesis which takes place, for example, in commercial butanol, ethanol, and penicillin fermentations contributes to the value of the residues recovered as feed adjuncts. A better understanding of those factors which control vitamin synthesis in such fermentations would not only make for greater uniformity of feed by-products but also offer the possibility of improving the potency of the more valuable constituents.

In the present review an attempt has been made to correlate available information on the content and synthesis of vitamins by microorganisms. For the most part, only quantitative data were considered. However, it should be emphasized that some of the data, particularly on the content of cells, were collected under diverse experimental conditions and therefore are not strictly comparable. They are intended primarily to illustrate the range of potencies to be expected rather than to provide a basis for delineating species or genera.

Intestinal synthesis and the like in which mixed cultures are known to be operative have been covered previously (Najjar and Barrett, 1945) and thus have been omitted from this discussion. Likewise, the occurrence in, and elaboration of unidentified animal growth factors by microorganisms, although well-recognized and of decided current interest, have not been included because of space limitations and the fragmentary nature of quantitative data.

II. THIAMINE

1. Content of Microorganisms

Listed in Table I are the thiamine potencies of various bacteria, yeasts, and molds. Within certain species, e.g., *Torula utilis*, there is considerable variation in content which in most instances is attributable to the use of different media and cultural conditions. Nevertheless, the

TABLE I
Thiamine Content of Microorganisms

	Dry cells, μg./g.	References
Bacteria		
<i>Aerobacter aerogenes</i>	11-15	315
<i>Azotobacter chroococcum</i>	33-96	149, 201
<i>Clostridium butylicum</i>	9.3	315
<i>Phytomonas tumefaciens</i>	12	180
<i>Propionibacterium pentosaceum</i>	0.4-6.2	271
<i>Proteus vulgaris</i>	21	315
<i>Pseudomonas fluorescens</i>	26	315
<i>Serratia marcescens</i>	27	315
Unidentified isolate from rat caecum.....	48	1
Yeasts		
<i>Candida arborea</i>	31.3-33.1	3
<i>Endomyces vernalis</i>	15-34	212, 322
<i>Hansenula suaveolens</i>	8.5	132
<i>Mycotorula lipolytica</i>	5.3	132
<i>Oidium lactis</i>	20.1-29.0	3
<i>Saccharomyces carlsbergensis</i>	31	322
<i>Saccharomyces ellipsoides</i>	38	322
<i>Saccharomyces cerevisiae</i>	29-90	3, 211, 272, 322
<i>Saccharomyces logos</i>	7.5-30	212
<i>Torula utilis</i>	6.2-52.8	71, 132, 156, 157, 205, 248, 272, 300, 314
<i>Willia anomala</i>	10-30	212
<i>Zygosaccharomyces pini</i>	39	322
Commercial bakers' yeast.....	9-40	176, 177, 211, 322
Commercial bakers' yeast (enriched).....	650-750	176, 177
Commercial foil yeast.....	80-150	176, 177
Commercial brewers' yeast.....	50-360	39, 71, 172, 266
Molds		
<i>Aspergillus oryzae</i>	18	251
<i>Fusarium lini</i> Bolley.....	20	326
<i>Fusarium graminearum</i>	5	326
<i>Penicillium notatum</i>	6.9	200
<i>Penicillium chrysogenum</i>	2.6	200

three groups of organisms exhibit the same range of values particularly when grown on essentially thiamine free substrates. No species is an unusually rich source of thiamine and only *Propionibacterium*, partially deficient for thiamine, is of exceptionally low potency.

As discussed in detail below, the thiamine which occurs in microorganisms arises from (a) total synthesis, (b) absorption from the medium, and (c) partial synthesis involving precursors. Absorption and partial synthesis are the more important from a quantitative viewpoint although other factors significantly influence synthesis and cell potencies.

2. Microbial Synthesis

Bacteria were early recognized as a source of B vitamins. Damon (1923) showed that vitamin B was elaborated by Pfeiffer's bacillus, *Mycobacterium phlei*, and *Mycobacterium mageritense*. A few years later Scheunert and Schieblisch (1927) demonstrated that *Bacillus vulgaris* cells, grown on a medium devoid of vitamin B, promoted the growth of pigeons maintained on a B-deficient diet. *Bacillus mycoides* and *Bacillus mesentericus* contained less of the growth-promoting principle than *B. vulgaris*. The ability of *B. vulgaris* to synthesize thiamine was confirmed subsequently by Guha (1932) and by Burkholder and McVeigh (1942).

Although many species remain to be surveyed, it is now assumed that most bacteria are capable of thiamine biosynthesis to meet their metabolic requirements. When cultivated on carbohydrate-nutrient salt mixtures, thiamine has been quantitatively determined in the cells of *Rhizobium trifolii* (West and Wilson, 1938), *Phytomonas tumefaciens* (McIntire et al., 1941), *Azotobacter chroococcum* (Lee and Burris, 1943) and in various intestinal bacteria (Burkholder and McVeigh, 1942). From more complex but thiamine-free substrates, thiamine has been demonstrated in the cells of *Aerobacter aerogenes*, *Serratia marcescens*, *Pseudomonas fluorescens*, *Proteus vulgaris*, *Clostridium butylicum* (Thompson, 1942), *Escherichia coli*, *Bacterium bifidum* (Reichelt, 1941), *Mycobacterium tuberculosis* (Lutz, 1947), and in various actinomycetes (Herrick and Alexopoulos, 1943). Another notable example of synthesis is that effected from wholly inorganic medium constituents by *Thiobacillus thiooxidans* (O'Kane, 1942).

Thiamine formation by bacteria varies with the strain of organism and its cultural history in addition to the medium and culture conditions. That bacterial cells which normally are heterotrophic for thiamine can be trained to synthesize their needs is indicated from the data of Silverman and Werkman (1939) with *Propionibacterium pentosaceum*. By acclimatization in the absence of thiamine cells increased in content

from 0.4 to 6.25 $\mu\text{g./g.}$ Presumably because they synthesize thiamine, other species of *Propionibacterium*, such as *P. freudenreichii*, grow equally well in its presence or absence (Tatum *et al.*, 1936). Quantitative differences among strains of *Mycobacterium tuberculosis* have also been observed by Lutz (1947) who found that human, avian, bovine, and BCG strains produced respectively, 19.45, 14.75, 10.04, and 7.75 $\mu\text{g. thiamine/g. dry cells.}$

Burkholder and McVeigh (1942) report appreciably higher thiamine values for intestinal bacteria than other investigators, in some instances for the same species. On a wet cell basis their figures for synthesis were as follows: *Escherichia coli* 115, *Bacillus vulgatus* 72, *Proteus vulgaris* 95, *Aerobacter aerogenes* 43, *Alkaligenes fecalis* 132, and *Bacillus mesentericus* 53 $\mu\text{g./g.}$ Total synthesis (cells and medium) for *A. aerogenes* and *P. vulgaris* were reported by Thompson to be only 19.9–26 and 21–23 $\mu\text{g./g. dry cells, respectively}$ (Thompson, 1942). Strain variation as well as culture conditions and the period of incubation may partially explain these differences. Genung and Lee (1944) reported that the thiamine content of the medium influenced the production of this vitamin by certain strains of *Escherichia coli*. This is a singular case since, generally within the limits of concentration usually encountered, the presence of a vitamin in the medium does not affect its synthesis.

Although relatively few studies have been carried out on the coupling of thiamine intermediates by bacteria, the work of Knight (1937) with *Staphylococcus aureus* demonstrates that this property is not restricted to yeasts and fungi which are discussed later. Likewise, certain bacteria tend to retain synthesized thiamine and may absorb additional quantities from solution. Scheunert and Schieblich (1927) noted that cells of *Bacillus vulgatus* possessed vitamin B activity whereas the cell-free medium was inactive. Similarly, with an unidentified organism isolated from rat caecal contents Abdel-Salaam and Leong (1938) showed that cells increased in thiamine content during the early hours of incubation and attained highest potencies at 24 hours. Thereafter, during a 9-day incubation period the level in the cells gradually decreased. It is of interest that these investigators offered the explanation confirmed by later studies (Hochberg *et al.*, 1945; Parsons *et al.*, 1945) that, due to the retentive ability of intestinal organisms for the vitamin B which they produce, no benefit is derived by the host maintained on a deficient diet.

In molasses medium Lee and Burris (1943) found that *Azotobacter chroococcum* cells became approximately three times as rich in thiamine as when propagated in sucrose medium. Since all the thiamine was concentrated in the cells it is probable that absorption from the molasses medium accounted for the difference in potency. However, not all

bacteria completely retain synthesized thiamine. Thompson (1942) found from about 10 to 60% of thiamine produced by various bacteria to be excreted into the medium within 24 hours.

That yeasts are capable of total synthesis of thiamine from carbohydrate-nutrient salts media was disclosed by Scheunert *et al.* (1939). They noted that *Torula utilis* contained thiamine after eleven continuous transfers in thiamine-free synthetic medium. Elaboration of thiamine on synthetic media has been observed as well with *Saccharomyces logos*, *Endomyces vernalis*, and *Willia anomala* (Pavcek *et al.*, 1938) and with *Saccharomyces cerevisiae* (Van Lanen *et al.*, 1942). On thiamine-free substrates, however, some species of yeasts have greater synthetic capacities than others. Livshits (1941) found *Torula utilis* and a strain of commercial brewers' yeast more productive than *Saccharomyces logos*, bakers' or distillers' yeast; whereas of several types valued by Pavcek *et al.* (1938), *Endomyces vernalis* yielded approximately double that obtained with other species. Some increase in the synthetic ability of yeasts might result, too, from continuous subculturing in the absence of thiamine according to Leonian and Lilly (1943).

From the reports of Fink and Just (1941a, 1942a), Lewis *et al.* (1944) and Odintsova (1941), it is clear that anaerobic conditions are more conducive than aerobic to thiamine production. The higher potencies of anerobic cells cannot be ascribed merely to enrichment through absorption since cells maintained anaerobically in the absence of thiamine also increase in potency (Maizel, 1946). Similarly Odintsova (1941) obtained values of 10 and 20 $\mu\text{g./g.}$ for *Torula utilis* grown aerobically and anaerobically respectively, in synthetic medium.

Yeasts grown on wood sugars and especially wood sugar stillage (Kurth and Cheldelin, 1946) are considerably lower in potency than those propagated on molasses perhaps owing either to enrichment by absorption of thiamine from molasses or to an inhibition of synthesis by substances contained in wood hydrolyzates. A relatively low incubation temperature and a pH of approximately 4.3 were observed by Pavcek *et al.* to enhance synthesis (1937, 1938). In media deficient in iron, thiamine production by *Torula utilis* was increased appreciably (Lewis, 1944).

Fluoride and compounds which react with carbonyl groups such as cyanide, sulfite, semicarbazide, and hydroxylamine reduced synthesis (Eppright and Williams, 1946). Pyrithiamine which interferes with the action of thiamine does not markedly influence its formation by microorganisms (Woolley and White, 1943).

Most lower fungi are autotrophic for thiamine, as is demonstrated by the studies of Fawns and Jung (1933) with *Aspergillus niger*, Scheunert and Schieblich (1936) and Sakurai (1940) with *Aspergillus oryzae*,

Penicillium glaucum, and *Mucor racemosus*, Carpenter and Friedlander (1942) with various fungi, and Vinson *et al.* (1945) with *Fusaria*.

Factors influencing thiamine synthesis by molds have received scant attention. *Fusarium lini* Bolley was observed to contain 20 $\mu\text{g.}$ while *F. graminearum* had a potency of only 5 $\mu\text{g.}$ thiamine/g. (Vinson *et al.*, 1945) indicating species differences. Apparently cultural practices also affect its production. With *F. lini* Bolley, cocarboxylase was produced in greater amounts with potassium nitrate than with ammonium sulfate as the source of nitrogen (Wirth and Nord, 1942).

Media low or absent in chlorides and maintained between 24 and 25°C. were reported to favor thiamine elaboration by *Aspergillus oryzae*. Supplements of nucleic acid and sodium urate were more satisfactory than ammonium nitrate (Wirth and Nord, 1942).

3. Synthesis from Precursors

That microorganisms are capable of utilizing portions of the molecule to synthesize thiamine was indicated in the investigations of Pavcek *et al.* (1937) wherein it was observed that thiamine which had been destroyed by heat was regenerated by bakers' yeast. In the same year Knight (1937) reported that pyrimidine and thiazole could replace thiamine in the nutrition of *Staphylococcus aureus* and in 1938 Schultz *et al.* announced the biological activity of these compounds for yeasts requiring thiamine. Following these demonstrations numerous studies which have been reviewed extensively elsewhere (Knight, 1945; Schopfer, 1943) were undertaken with various fungi, protozoa, and higher plants.¹

As a result of their investigations with yeasts, Schultz *et al.* (1941) developed a commercial method for increasing the thiamine content of bakers' yeast. In this process the pyrimidine and thiazole moieties were supplied to yeast mashes during propagation to provide cells varying from 300 to 1200 $\mu\text{g.}$ thiamine/g. While bakers' strains couple these intermediates more efficiently than do other yeasts, Van Lanen *et al.* (1942) and Fink and Just (1942b) found that *Saccharomyces carlsbergensis*, *Saccharomyces ellipsoidus*, *Endomyces vernalis*, *Zygosaccharomyces pini*, *Torula utilis*, *Oidium lactis*, and *Aspergillus oryzae* also are active. With bakers' yeast utilization of pyrimidine and thiazole proceeds almost quantitatively until the cells attain a vitamin potency of 700 to 800 $\mu\text{g./g.}$ above which the efficiency of conversion decreases sharply. When supplied with comparable amounts of intermediates the other yeasts ranged in efficiency from 6 to 65% (Van Lanen *et al.*, 1942).

The coupling of intermediates is markedly enhanced by aeration, a low temperature of incubation, and by a factor(s) contained in spent

¹ Cf. the review of Bonner and Bonner in this volume.

wort (Van Lanen *et al.*, 1942). Whether the factor(s) in wort is similar to or identical with that found by Kidder and Dewey (1942) to be essential for the coupling of pyrimidine and thiazole by protozoa is not known.

When provided with one intermediate in the absence of the other, yeasts vary in their ability to complete the synthesis. Schultz *et al.* (1941) found Fleischmann yeast strain 189 to be capable of synthesizing some thiamine from pyrimidine, while Van Lanen *et al.* (1942) Odintsova (1943) and Fink and Just (1942b and c) reported the formation of thiamine from thiazole but found negligible synthesis with pyrimidine alone.

In general, commercial yeasts are more exacting than most other fungi, e.g., *Phycomyces* species (Knight, 1945; Schopfer, 1943), with regard to the intermediates which they can utilize for growth or convert quantitatively to thiamine. No thiazoles other than 4-methyl, 5- β -hydroxyethylthiazole and the same in which the β -hydroxyl group has been replaced by an acyl group (Mead and Lee, 1943) have been found satisfactory. The patent of Harrison (1944), however, covers the use of thiazoles containing in the β -hydroxyl position either hydrolyzable or otherwise replaceable substituents. Yeast, but not *Staphylococcus aureus*, also is capable of converting *l*- α -amino-4-methyl-5-thiazole-propionic acid into 4-methyl-5- β -hydroxyethylthiazole (Harrington and Moggridge, 1940).

The requirement for the pyrimidine moiety appears to be equally specific, with only certain substitutions in the 5 position permissible. Of these aminoethyl or hydroxymethyl (Ashida, 1942; Fink *et al.*, 1942), cyano (Harrison, 1944), aminomethyl (Fink and Just, 1942c), and thioformyl or ethoxymethyl (Schultz *et al.*, 1941) groups can be converted by various yeasts to thiamine through methylenic linkage with the nitrogen of thiazole.

4. Absorption by Microorganisms

That thiamine from the medium can be concentrated within the cells of microorganisms was first detected by Eijkman *et al.* (1922) who noted that both bakers' and brewers' yeasts removed the antineuritic principle from solution. Scheunert and Shieblieh (1935) reached the same conclusion after noting that beer contained only small amounts of vitamin B whereas, when fortified after fermentation had been completed and the yeast separated no subsequent destruction occurred. Pavcek *et al.* (1936) and Fischer (1938) confirmed the behavior of yeasts with respect to thiamine and Schopfer (1943) extended the observation to *Phycomyces*. From these reports and their subsequent elaboration industrially feasible methods evolved for concentrating thiamine from natural products and

simultaneously preparing enriched yeasts. In one of these processes (Gorcica and Levine, 1942) thiamine was first extracted from such materials as wheat germ, rice polishings, or malt sprouts after which sugar was added to yield the desired amount of yeast of predetermined thiamine potency and the medium was fermented. In another method, Schultz *et al.* (1942) similarly proposed the enrichment of yeast employing crystalline thiamine. Yeasts can be enriched in this manner to give potencies of 650 $\mu\text{g. thiamine/g.}$ with high efficiency (Fink and Just, 1941b; Schultz *et al.*, 1942) and cells have been fortified as high as 6260 $\mu\text{g./g.}$ with lower efficiencies (Van Lanen *et al.*, 1942).

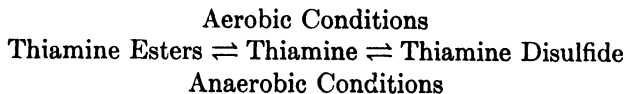
The abstraction of thiamine from solution by yeast takes place both aerobically and anaerobically in the presence of fermentable substrate but poorly under anerobic conditions in the absence of substrate. Fink and Just (1941b) noted that *Torula* yeast exhibits maximum absorption when introduced into 20% glucose concentrations which restrict fermentation. High temperatures, galactose or maltose (Fink and Just, 1941b), and alcohol (Sperber and Renvall, 1941) also enhance uptake while xylose and lactose are without effect. When the permeability of the cells is disturbed, thiamine uptake is decreased (Fink and Just, 1941b). Actually, in the presence of small amounts of salts, especially multivalent ions, absorbed thiamine may be excreted (Sperber, 1942).

Based on a similarity of the action of various inhibitors and on the optimum pH for absorption (3.5–4.0), Sperber (1942) has advanced the theory that yeast phosphatase is concerned with thiamine uptake. Iodacetate, azide, and fluoride completely inhibit absorption while cyanide is partially inhibitory. Both absorption and phosphatase activity likewise are retarded by 2-methyl, 4-amino, 5-methylamino-pyrimidine.

Thiamine in the cells of microorganisms may be present in the free state, as the mono- or diphosphates, or as thiamine disulfide, the proportion of each form varying with the history of the cells. Hochberg *et al.* (1945) found that phosphorylated thiamine constituted from 17 to 93% of the thiamine found in commercial yeasts, the lower values being associated with thiamine enriched samples. Fink and Just, with enriched cells of *Torula utilis*, report figures up to 97% for the phosphorylated form (Fink and Just, 1942a). Upon drying (Hochberg *et al.*, 1945) or aging (Teyssie, 1945) yeast phosphatases are liberated which in turn decrease the amount of phosphorylated thiamine in the cells.

Myrbäck and Vallin (1944), from an examination of the thiamine and cocarboxylase potencies of various yeasts, have concluded that in cells propagated anaerobically, such as brewers' yeast, cocarboxylase predominates. In aerobic yeast, on the other hand, thiamine is largely

present in the form of the disulfide which results from cleavage of the thiazole ring and oxidation of the sulfur. Thiamine disulfide does not respond to the usual assay procedures. They suggest the following transition:



Since thiamine esters are absent in strongly aerated cells and fermentative activity is correspondingly suppressed the above has been suggested as an explanation of the Pasteur effect. However, considerable evidence to support the conclusion that thiamine disulfide exists in insignificant amounts in yeast cells has been offered by Schonberg and Sperber (1945).

5. Thiamine Inactivation

Upon continued incubation especially under aerobic conditions, microorganisms destroy thiamine both in the cells and in the medium (Fink and Just, 1941a, 1942a; Kavanagh, 1942; Lewis, 1944; Van Lanen *et al.*, 1942). The enzymatic degradation of thiazole (Bonner and Buchman, 1939) and thiamine pyrophosphate (Westenbrink and Veldman, 1942) in plant tissues and yeast cells, respectively, already have been described. Thiamine is inactivated also by aquatic animals through cleavage of the methylene bridge (Krampitz and Woolley, 1944). Whether inactivation of thiamine by microbial cells is attributable to (a) enzymes similar to those mentioned above, (b) metabolic degradation such as has been noted in the case of the codehydrogenases, or (c) conversion to disulfide or other inactive forms is a subject for speculation. Nevertheless it is readily apparent that losses do occur and are one of the factors determining the thiamine potencies of various microorganisms.

III. RIBOFLAVIN

Riboflavin synthesis has been demonstrated qualitatively in a wide variety of microorganisms through growth studies. These have been adequately reviewed elsewhere (Knight, 1945; Peterson and Peterson, 1945). Table II summarizes typical results of quantitative studies of microbial biosynthesis. Of the bacteria none has been reported which exceeds the capacity of *Clostridium acetobutylicum* to produce this vitamin. Among the molds, few have been examined, but *Aspergillus niger* has been reported to excrete relatively large amounts of the vitamin. While commercial yeasts have long been considered rich sources other yeasts such as *Candida guilliermondia*, *C. flareri*, and the Ascomycetes,

Eremothecium ashbya and *Ashbya gossypii*, are capable of producing prodigious amounts.

In spite of the attention which has already been directed toward a fuller understanding of the factors affecting biosynthesis of riboflavin, only limited progress has been made. Some of the factors influencing synthesis by several important microorganisms are discussed below.

TABLE II
Riboflavin Content of Microorganisms and Whole Cultures

	Dry cells, μg./g.	Medium, μg./ml.	References
Bacteria			
<i>Aerobacter aerogenes</i>	3.7	236
<i>Acetobacter suboxydans</i>	8.5	319
<i>Azotobacter vinelandii</i>	305-350	149
<i>Bacillus pasteurianum</i>	15	256
<i>Clostridium acetobutylicum</i>	50 ¹	183
<i>Clostridium acidurici</i>	14.3	18
<i>Clostridium butyricum</i>	130	256
<i>Lactobacillus delbrückii</i>	115	256
<i>Mycobacterium smegmatis</i>	8600	57.5	179
<i>Mycobacterium tuberculosis</i>	150-200	28, 33
<i>Phytomonas tumefaciens</i>	2.9	180
Yeasts			
<i>Ashbya gossypii</i>	1000	302
<i>Candida arborea</i>	46.5-69.5	3, 272
<i>Candida flareri</i>	216	303, 304
<i>Candida guilliermondia</i>	157	303, 304
<i>Eremothecium ashbyii</i>	1800	218a
<i>Hansenula suaveolens</i>	54	132
<i>Mycotorula lipolytica</i>	59	132
<i>Oidium lactis</i>	39.9-55.0	3, 272
<i>Saccharomyces cerevisiae</i> , (primary).....	39.1-75	3, 199, 272
<i>Saccharomyces cerevisiae</i> (brewers').....	36-42	199
<i>Torula utilis</i>	26-62	3, 132, 157, 272
Molds			
<i>Aspergillus niger</i>	5	147
<i>Penicillium chrysogenum</i>	39.8-47.5	301

1. Synthesis by Bacteria

Of all the B vitamins, microbial synthesis of riboflavin has been encountered most frequently. Members of the following genera have been reported to produce from 0.02 to 0.36 μg./ml. when cultured in riboflavin-free media: *Escherichia*, *Aerobacter*, *Klebsiella*, *Serratia*, *Proteus*, *Pseudomonas*, *Alkaligenes*, *Achromobacter*, *Chromobacterium*,

Flavobacterium, *Propionibacterium*, *Micrococcus*, *Staphylococcus* and *Bacillus* (Tittsler and Whittier, 1941). *Clostridium acidu-urici* (Barker and Peterson, 1944) grown in synthetic medium with uric acid as sole source of nitrogen produced 14.3 $\mu\text{g./g.}$ dry cells. When cultured aerobically in distillers' thin stillage, *Aerobacter aerogenes* and *A. cloacae* multiplied the riboflavin content fivefold and threefold, respectively (Novak *et al.*, 1943). A fluorescent pigment formed by *Corynebacterium* also has been identified as riboflavin (Crawe, 1939).

Clostridium acetobutylicum. Riboflavin synthesis by this organism in the course of the butanol fermentation has been of industrial importance for several years and serves as an excellent example of the development of valuable by-products from fermentation residues. Recovery of wastes from this fermentation followed the observation by Miner (1940) that mashes fermented with *Cl. acetobutylicum* contained much more riboflavin than the original mash components, assaying from 25 to 62.5 $\mu\text{g./g.}$ on a dry basis.

In grain fermentations the formation of an intense yellow pigment had been noted in 1927 by Weyer and Rettger, however, they attributed it to the solubilization of zein by the neutral solvents formed. Undoubtedly, these workers had observed the biosynthesis of this vitamin before it had been isolated or identified. At about the same time as Miner, others identified the yellow pigment which appeared in butanol fermentations of rice with the then newly-characterized vitamin (Yamasaki, 1939, 1943; Yamasaki and Yoshitome, 1938).

Following the demonstration that animal feeds required supplemental riboflavin, efforts were made to improve biosynthesis in commercial butanol fermentations. From various studies which have been made using different techniques it has been established that optimum riboflavin formation occurs only when the iron concentration of the medium is maintained between 1 and 3 p.p.m. This level has been determined by (a) determination of iron in various mashes, (b) the use of agents to render iron biologically unavailable, and (c) the addition of iron to deficient media. Whereas iron is very critical to riboflavin formation, only high concentrations adversely affect solvents production. By limiting the iron content of corn mashes, Arzberger (1943) obtained more than 3000 $\mu\text{g.}$ riboflavin/g. dry residue. He found (Table III) that cobalt as well as iron inhibited appreciably while salts of zinc, copper, and lead were much less active.

In commercial butanol fermentations for the production of riboflavin concentrates, significant contamination of mashes with iron arises from the grain, steam and water supply, and from stillage when the latter is added to supply additional nutrients. Walton (1945) demonstrated

TABLE III
*Effect of Metals on Riboflavin Synthesis by Clostridium acetobutylicum*¹

Compound added	Concentration, mg./l.	Solvent yield, % of dry corn	Riboflavin based on	
			Original corn, μg./g.	Dried filtrate, μg./g.
None.....	26.4	508	2720-3150
Ferrous sulfate.....	3.2	26.6	163	810
	32.0	27.8	39	197
	320.5	28.0	15	69
	3205.0	24.3	31	145
	32050.0	3.2	38	33
Cobaltous acetate.....	3.2	27.7	105	620
	32.0	27.9	75	242
	320.5	12.8	32	64
Cupric acetate.....	3.2	31.0	...	3180
	32.0	31.0	387	2032
	320.5	6.7	25	41
Zinc acetate.....	3.2	27.6	603	3430
	32.0	29.4	539	2945
	320.5	29.2	462	2440
	3205.0	4.0	34	55
Lead acetate.....	3.2	29.6	460	2500
	32.0	29.8	407	2295
	320.5	28.4	422	2110
	3205.0	5.3	26	39

Mash composed of 5% cleaned, dry corn.

¹ From: Arzberger, C. F., U. S. Patent 2,326,425, 1943.

that rice which is relatively low in iron was an excellent supplement to corn and led to high yields of riboflavin.

Yamasaki (1941, 1942) also recognized the significance of iron in butanol fermentations and observed that the elaboration of riboflavin was increased by adding from 0.1 to 2% calcium carbonate or calcium acetate during the first 7 hours of fermentation. This effect is probably due to the absorption of iron by these salts since a similar principle was employed by Steinberg (1935, 1938, 1939) to prepare media deficient in trace elements. By adding from 0.005 to 0.05% sodium sulfite to grain mashes, Legg and Beesch (1945) substantially increased riboflavin synthesis.

In a study of various aerobic and anaerobic bacteria, Saunders and McClung (1943) noted that addition of traces of iron as ferrous sulfate stimulated riboflavin production in the case of four of five strains of *Cl. acetobutylicum*. These results should not be considered at variance

with those above since the iron content of grain and water differ with the locality. Also, slightly different optimal iron levels probably exist for different strains of the species. Saunders and McClung, confirming Rodgers (1942), noted that the stimulatory effect of iron appeared to be limited to strains of *Cl. acetobutylicum* because *Cl. roseum*, *Cl. felsineum* and other pigmented anaerobes, including certain yellow butyric types, did not respond to the iron supplements although these latter anaerobes do not produce large amounts of riboflavin.

Some strains of *Cl. acetobutylicum* actively ferment lactose with the elaboration of substantial amounts of riboflavin. Whey or skim milk, however, do not support vigorous or complete fermentation unless iron is added. Meade *et al.* (1945) found that whey fermented after adjustment of the iron content to 0.5 to 4.5 p.p.m. gave yields of riboflavin of 50 or more $\mu\text{g.}/\text{ml.}$ Within these limits of iron concentration the actual optimum for riboflavin synthesis was found to vary with the pH, and the acids used (Pollard *et al.*, 1947; Rogers *et al.*, 1947). With iron controlled, the addition of 0.5 to 2.0% xylose to whey was observed to increase further the synthesis of this vitamin (Meade *et al.*, 1947). As with grain mash, iron is much more critical to riboflavin synthesis than to solvents production.

Hickey (1945, 1947) has investigated the ability of organic compounds, frequently used in analytical procedures, to render iron biologically unavailable to *Cl. acetobutylicum*. Of these cupferron, 7-iodo, 8-hydroxyquinoline, 5-sulfonic acid, and the anions, cyanide, oxalate, thiocyanate, sulfide, and citrate were ineffective. However, 2,2'-dipyridine was employed successfully in corn mash to which iron had been added. Hickey observed that 9.5 $\mu\text{g.}$ of dipyridine were required to bind 1 $\mu\text{g.}$ of ferrous iron whereas stoichiometrically, 8.4 $\mu\text{g.}$ are needed. The slight excess was thought necessary to suppress dissociation of the iron complex. Dipyridine did not increase riboflavin production by two molasses fermenting strains of *Cl. acetobutylicum*. This was attributed to peculiarities of these strains or to inhibition by substances other than iron. On the other hand, iron may occur in a form not readily reactive with dipyridine, although biologically available to the organism.

An explanation of the action of iron in *Cl. acetobutylicum* fermentations has been proposed by Leviton (1946). He noted that riboflavin, although stable in pure solutions to hydrogen peroxide, is readily decomposed by dilute solutions of this reagent when traces of ferrous iron are present. Between iron levels of 0.018 and 0.054* mg. atoms/l. (about 1 to 3 p.p.m. of iron) the rate constants for riboflavin destruction rose from 2.3 to

* Leviton (personal communication) corrected to 0.1 those iron concentrations given originally (1946).

18.9%/hour. It will be recalled that the higher level of iron is approximately that which is critical to riboflavin accumulation in cultures of the butyl anaerobes. Leviton found further that catalase, potassium iodide, and sulfite which accelerate the decomposition of H_2O_2 stabilized riboflavin. This evidence along with the known functions of enzymes in which flavin-adenine-phosphoric acid comprise the prosthetic group has been put forth to support the conclusion that H_2O_2 is produced by *Cl. acetobutylicum* although its formation has not been demonstrated. Whether the action of iron can be explained (a) by its capacity to destroy riboflavin when in combination with H_2O_2 as described above, or (b) by permitting an alternative and preferred respiratory mechanism to function (Hickey, 1945), the involvement of iron porphyrin catalysts is indicated. This suggests the need to reinvestigate the generally accepted belief that all obligate anaerobes are devoid of these systems.

Considerable quantities of riboflavin likewise are produced by *Mycobacterium smegmatis* (Mayer and Rodbart, 1946). Unlike *Cl. acetobutylicum* synthesis is not affected by iron. In Long's medium, yields of more than 20 $\mu\text{g.}/\text{ml.}$ were formed in 8–12 days. When glycine replaced asparagin in the above medium, both growth and vitamin synthesis were depressed. Other amino acids were without effect, but with nitrate or ammonium salts as the only nitrogen source potencies up to 57 $\mu\text{g.}/\text{ml.}$, equivalent to 8600 $\mu\text{g.}/\text{g.}$ dry cells, were secured. Glycerol in the original medium was replaceable by levulose but not glucose although the latter supported good growth.

Mycobacterium tuberculosis produced riboflavin but in somewhat lesser amounts (Boissevain *et al.*, 1938; Rohner and Roulet, 1939), cultures assaying 2.9 $\mu\text{g.}/\text{ml.}$ having been obtained.

2. Synthesis by Yeasts

The general yeasts of commerce such as those for food and baking purposes contain from 40 to 85 $\mu\text{g.}$ riboflavin/g. The quantity withheld by the cells during propagation is apparently rather constant whereas variable amounts are released into the medium. Massock (1943) and Van Lanen (unpublished) were unable to alter appreciably either riboflavin synthesis or retention by *Saccharomyces cerevisiae* by varying culture conditions. However, according to Pett (1935) the content of *S. cerevisiae* can be raised by culturing in the presence of compounds such as KCN, cysteine, and pyridine which affect the activity of the iron-containing catalysts. It was observed further by Pett that aerobic conditions lower the riboflavin content of yeast cells. This has been substantiated by Singh *et al.* (1947) who found that cells produced without aeration or agitation (low cell crop) contained 108 $\mu\text{g.}$ while those aerated (high cell crop) contained 60–65 $\mu\text{g.}$ riboflavin/g.

Iron also influences riboflavin synthesis by *Torula utilis* but less markedly than with some of the organisms discussed above. Lewis (1944) found that cells propagated in synthetic medium in the absence of iron were slightly higher in content than when iron was supplied. Of interest in connection with this organism is the fact that *T. utilis* was higher in riboflavin content from synthetic medium than from fruit juice (Lewis *et al.*, 1944) or other media (National Research Council, 1943).

In 1943 and subsequently Burkholder described an interesting species, *Candida guilliermondia*, which is capable of outstanding synthesis of riboflavin (1943a and b, 1944). In a medium composed of 2% glucose, 0.2% ammonium sulfate and other inorganic salts yields up to 40 $\mu\text{g.}/\text{ml.}$ were produced, and an increase of approximately 50% resulted when certain amino acids were added. Other investigators found this organism to vary considerably in riboflavin-producing ability, the explanation for which was disclosed by Tanner *et al.* (1945, 1947b) who established that iron again was the major controlling factor in this fermentation. The optimum iron level for riboflavin synthesis, however, was found to be in the neighborhood of 0.005 to 0.01 p.p.m. compared with 1-3 p.p.m. for *Clostridium acetobutylicum*. When precautions were taken to regulate the iron concentration, yields up to 216 $\mu\text{g.}$ riboflavin/ml. were secured with *Candida* species. Waring and Werkman (1943) have shown that in cells of *Aerobacter indologenes* produced on nutrient solutions depleted of iron, catalase activity was greatly reduced and cytochrome could not be demonstrated.

Among the *Ascomycetes* are two yeastlike organisms, *Eremothecium ashbyii* and *Ashbya gossypii*, which are capable of synthesizing extraordinary amounts of riboflavin. These organisms, both plant pathogens, have been described by Guilliermond (1928, 1936). They have many characteristics in common including the formation of short, fine-structured mycelia but can be differentiated morphologically principally by the shape of their ascospores (Guilliermond, 1936). In the course of studying *E. ashbyii*, Guilliermond *et al.* (1935) noted that a greenish-yellow pigment was formed on Gorodkowa or Sabouraud agar and crystals of the pigment frequently were observed in cell vacuoles. Physical and chemical properties indicated a similarity to riboflavin and confirmation of its identity with this vitamin was obtained by Raffy (1937) and Miramanoff and Raffy (1938a and c).

The French workers obtained appreciable synthesis of riboflavin with *Eremothecium ashbyii* (112 $\mu\text{g.}/\text{g.}$ agar medium and 159 $\mu\text{g.}/\text{ml.}$ liquid medium) but incubation periods as long as 24 days were necessary (Raffy and Fontaine, 1939; Renaud and Lachaux, 1944, 1945). The requirement for aerobic conditions, likewise, was recognized (Raffy,

1939), a feature which has been extensively elaborated upon by later investigators (Foster, 1947; Piersma, 1945; Rudert, 1945; Stiles).

Schopfer (1944) called attention to biochemical differences separating *Eremothecium ashbyii* from *Ashbya gossypii* and confirmed Guilliermond *et al.* (1935) with regard to the fact that only trace amounts of riboflavin are formed by the latter organism. A further contrast has been drawn on the basis of growth requirements since *E. ashbyii* requires accessory factors over and above thiamine, biotin, and inositol which are needed by both organisms (K'gl and Fries, 1937; Robbins and Schmidt, 1939). The accessory substance for *E. ashbyii* is contained in norite-treated peptone and is partially replaceable by leucine and arginine (Schopfer and Guilloud, 1945a).

Despite the definitely-established need for aerobic conditions for maximum riboflavin elaboration by *Eremothecium ashbyii*, submerged, aerobic techniques were not applied until visualized in 1941 by Mayer (1946). By this method yields of 80 $\mu\text{g.}/\text{ml.}$ were secured and the commercial possibilities of this organism were indicated. Several patents followed employing processes basically related in their adaptation to submerged conditions, but utilizing as the fermentable substrate various proteinaceous substances with carbohydrates (Foster, 1947; Piersma, 1945; Stiles) or lipides (Rudert, 1945). Yields as high as 400 $\mu\text{g.}/\text{ml.}$ in 160 hours were reported. Moore and de Becze (1947), by fermenting supplemented ethanol stillage with *E. ashbyii*, prepared residues containing 4000 $\mu\text{g.}$ of riboflavin/g. on a dry basis.

In contrast to earlier reports (Guilliermond, 1935; Schopfer and Guilloud, 1945b, *Ashbya gossypii* has been shown to be capable under certain conditions of riboflavin synthesis of the same order of magnitude as *Eremothecium ashbyii*. Wickerham *et al.* (1946) obtained potencies up to 381 $\mu\text{g.}/\text{ml.}$ in a medium of glucose, peptone, and yeast extract. Tanner and Van Lanen (1947a) discussed the factors influencing vitamin production by this organism and reported yields of 1000 $\mu\text{g.}/\text{ml.}$ in a medium composed of 0.5% corn steep liquor solids, 0.5% animal tankage or peptone, and 2-4% glucose.

Although neither *Eremothecium ashbyii* nor *Ashbya gossypii* is affected by iron in the concentrations normally encountered, elaboration of the vitamin by the latter organism is markedly inhibited by certain substances, probably organic in nature, which occur in various natural products.

3. Synthesis by Molds

The formation by *Aspergillus niger* in Raulin's solution of a water-soluble yellow pigment with fluorescent properties and subsequently

characterized as riboflavin has been described in a series of papers by Lavollay and Laborey (1937, 1941). Maximum yields, in the order of 5 $\mu\text{g.}/\text{ml.}$ resulted when the medium contained no more than 0.4 mg. % of a magnesium salt. Ascorbic acid suppressed synthesis almost completely. Knobloch and Sellmann (1941) reported that numerous strains of *A. niger* elaborate a flavin-type pigment, notably when nitrogen is supplied as magnesium nitrate.

Peltier and Borchers (1947) have made a study of the ability of molds to synthesize riboflavin when cultured on wheat bran. All of more than two hundred isolates tested produced some riboflavin with certain *Aspergilli* and *Fusaria* giving the highest yields. One strain of *Aspergillus* increased the bran from an initial value of about 3 $\mu\text{g.}/\text{g.}$ to 58 $\mu\text{g.}/\text{g.}$

IV. PANTOTHENIC ACID

The term pantothenic acid was coined to denote the universal distribution of a substance recognized in 1933 to have remarkable growth-promoting action for certain strains of *Saccharomyces cerevisiae* (Williams *et al.*, 1933). Even at this early date, synthesis by *Aspergillus niger* and *Bacillus subtilis* had been observed.

The function of pantothenic acid has only recently been elucidated with the demonstration that it is a component of coenzyme A. This coenzyme functions in the second or acetate phase of pyruvate oxidation in which acetyl phosphate is formed as well as in other acetylation reactions (Lipmann and Tuttle, 1945). Coenzyme A contains approximately 11% pantothenic acid or 0.7 $\mu\text{g.}$ per enzyme unit (Lipmann *et al.*, 1947a, 1947b). That pantothenic acid might be concerned with pyruvate oxidation was suggested by Dorfman *et al.* in 1942 and confirmed by Hills (1943).

Coenzyme A has been found in all microorganisms examined including species of *Lactobacillus*, *Propionibacterium*, *Escherichia*, *Proteus* and *Clostridium* (Novelli and Lipmann, 1947a). With *L. arabinosus* pantothenic acid in the form of coenzyme accounted for 90% of the total in the cells. Digestion with clarase and papain, a conventional procedure for liberating pantothenic acid, hydrolyzes the coenzyme slowly. Better success has been reported using a phosphodiesterase combined with an unidentified enzyme from liver (Lipmann and Kaplan, 1946; Lipmann *et al.*, 1947a). The requirement for both enzymes suggests that at least two linkages bind pantothenic acid in the coenzyme.

1. Synthesis by Microorganisms

Table IV summarizes the pantothenic acid contents of those organisms which have been studied quantitatively. It may be seen that

bacteria vary appreciably both with regard to the amounts produced and the degree of excretion, with *Aerobacter aerogenes* cultured anaerobically being the richest.

TABLE IV
Pantothenic Acid Content of Cells and Whole Cultures

	Dry Cells, μg./g.	Total syn- thesized, μg./g. dry cells	References
Bacteria			
<i>Aerobacter aerogenes</i> (aerobic).....	140	780	315
<i>Aerobacter aerogenes</i> (anaerobic).....	340	750	315
<i>Azotobacter vinelandii</i>	152-184	149
<i>Clostridium butylicum</i>	93	318	315
<i>Phytomonas tumefaciens</i>	41	180
<i>Proteus vulgaris</i>	100	100-130	315
<i>Pseudomonas fluorescens</i>	91	311	315
<i>Serratia marcescens</i>	120	172	315
Yeasts			
<i>Saccharomyces cerevisiae</i> (aerobic).....	118	176
<i>Saccharomyces cerevisiae</i> (anaerobic).....	198	176
<i>Hansenula suaveolens</i>	180	132
<i>Torula utilis</i>	86-180	132, 157, 199
Commercial foil yeast.....	120-180	176
Commercial bakers' yeast.....	180-330	176
Commercial brewers' yeast.....	100	199
Debittered brewers' yeast.....	72-86	199
Fungi			
<i>Penicillium chrysogenum</i>	107-140	301
<i>Penicillium chrysogenum</i>	201-212	200

Results of analysis of commercial yeasts show that foil yeast is significantly lower in pantothenic acid than bakers' types. The latter are differentiated from foil yeast by having a higher nitrogen content and higher activity. Anaerobically-propagated yeasts were found to have nearly double the level of aerobic cells (Massock and Baldwin, 1943) which is similar to experience with *A. aerogenes* (Thompson, 1942). That this does not hold for brewers' yeasts which are low in pantothenic acid is probably attributable to the fact that drying and debittering processes effect appreciable destruction.

Penicillium chrysogenum, one of the few molds studied synthesizes relatively large quantities of pantothenic acid. During fermentation of corn steep liquor-lactose medium this organism increased the level in the medium from 1.8 to nearly 18 μg./ml. (Tanner *et al.*, 1945). That

found only in the mycelium ranges from 108 to 200 $\mu\text{g./g.}$ (Newell *et al.*, 1947; Tanner *et al.*, 1945).

Whereas synthesized pantothenic acid is generally excreted to a considerable extent, absorption is accomplished and the absorbed vitamin is converted to coenzyme A by species which require an external source (Novelli and Lipmann, 1947b).

2. *Synthesis from Precursors*

Prior to the full elucidation of the structure of pantothenic acid, the ability of β -alanine to replace it in the growth of certain organisms had been observed. Subsequently, it was established that numerous organisms can accomplish the synthesis of pantothenic acid when provided with either β -alanine (Mueller, 1933; Mueller and Klotz, 1938) or pantoyl lactone (Ryan *et al.*, 1945; Underkofler *et al.*, 1943; Woolley, 1939). The promotion of pantothenic acid formation by *Saccharomyces cerevisiae* by supplementing the medium with β -alanine was the subject of a patent by Schultz *et al.* (1945). Most of the lactic acid bacteria are more exacting, however, and thus are unable to utilize these compounds (Cheldelin *et al.*, 1945; Snell *et al.*, 1938).

A cell-free system prepared from yeast which is capable of coupling β -alanine and pantoyl lactone has been described by Wieland and Moller (1941). Ammonium ions activated the system, 0.1 *M* producing a maximum response (Wieland and Moller, 1942).

Kuhn and Wieland (1942) have speculated that biosynthesis of pantothenic acid might stem from valine and aspartic acid as follows: Valine by oxidative deamination is convertible to dimethylpyruvic acid which may then be condensed with formaldehyde to α -keto- β , β -dimethyl- γ -hydroxybutyric acid. The latter readily undergoes lactonization and upon reduction gives α , γ -dihydroxy- β , β -dimethylbutyric acid which yeast can combine with β -alanine to give pantothenic acid. Aspartic acid could give rise to β -alanine through decarboxylation (Kuhn and Wieland, 1942; Ravel and Shive, 1946).

V. NIACIN

1. *Content of Microorganisms*

Compared with the tissues of higher plants and animals, microorganisms are not only richer in niacin but, despite fundamental differences in metabolism, they show remarkable uniformity. This may be taken as a reflection of the importance of this vitamin and corresponding coenzymes to their general economy. Analogous with thiamine, the

synthesis, absorption, excretion, and degradation of niacin have been shown to be operative in microbial cells and, as elaborated upon below, each functions in determining cell potencies. In view of the dynamic state which exists within the cell with respect to this vitamin, the degree of uniformity in potency is all the more noteworthy.

In Table V, values are given for the niacin content of various bacteria, yeasts, and molds. It is evident that, while marked by constancy in content, there are variations both within and between species. From

TABLE V
Niacin Content of Microorganisms and Culture Liquors

	Dry cells, μg./g.	Total syn- thesized in cells and medium, μg./g. dry cells	References
Bacteria			
<i>Aerobacter aerogenes</i> (anaerobic).....	200	630	315
<i>Aerobacter aerogenes</i> (aerobic).....	240	258	315
<i>Azotobacter chroococcum</i>	590	149
<i>Clostridium butylicum</i>	250	1930	315
<i>Proteus vulgaris</i>	250	330	315
<i>Pseudomonas fluorescens</i>	210	560	315
<i>Serratia marcescens</i>	240	470	315
Yeasts			
<i>Candida arborea</i>	157-580	3, 272
<i>Candida guilliermondia</i>	515	320
<i>Hansenula suaveolens</i>	590	132
<i>Monilia murrmanica</i>	272-375	223
<i>Mycotorula lipolytica</i>	600	132
<i>Oidium lactis</i>	195-248	3, 272
<i>Saccharomyces cerevisiae</i> (anaerobic).....	190	320
<i>Saccharomyces cerevisiae</i> (aerobic).....	585	320
<i>Saccharomyces cerevisiae</i> (enriched).....	2930	176
<i>Torula lactosa</i>	447	320
<i>Torula utilis</i>	210-535	39, 70, 132, 300, 314, 320
Commercial foil yeast.....	180-400	177
Commercial bakers' yeast.....	200-700	39, 70, 132, 177, 199, 266
Commercial brewers' yeast.....	320-1000	39, 172, 266, 300, 327
Molds			
<i>Penicillium chrysogenum</i>	150-212	200, 301
<i>Penicillium notatum</i>	180	200

the evidence available to date, these differences can be ascribed to environmental factors and to strain characteristics which affect both synthesis and the retentive properties of organisms for this vitamin. Except for *Azotobacter chroococcum*, the bacteria and molds which have been studied are lower in potency than the majority of the yeasts. Certain bacteria synthesize appreciable amounts of niacin but, as shown later, it is largely excreted into the medium. Yeasts propagated anaerobically become enriched in niacin when this vitamin is present in the medium but are of low potency when it is omitted. (Compare brewers' yeast with *Saccharomyces cerevisiae* grown aerobically and anaerobically in synthetic medium.) These and similar data substantiate the view that synthesis is limited while absorption is unaffected or improved under anaerobic conditions. Certain organisms such as *Candida arborea* have been found to vary in niacin potency depending upon the substrate whereas *Oidium lactis* is uniformly low. Explanation for these differences may reside in the limited ability of the former species to synthesize and of the latter to accomplish either synthesis or absorption. Several types of yeasts grown in molasses-malt extract medium have been shown (Van Lanen, 1946) to vary in niacin content with *Candida guilliermondia* and a strain of distillers' yeast giving the highest potencies. While all lowered the level in the medium, presumably through absorption, with only the two above species was the final niacin in cells and medium equivalent or greater than that initially present. Despite synthesis which undoubtedly occurred, it is clear then, that destruction took place and in most instances exceeded the rate of synthesis.

2. Synthesis of Niacin

Complete synthesis of niacin has been demonstrated by microorganisms cultivated on inorganic carbon and salts (O'Kane, 1942), sucrose, atmospheric nitrogen and salts (Lee and Burris, 1943), acetic acid, inorganic nitrogen and salts (Fink and Just, 1939) as well as from various other synthetic preparations.

Although produced from simple carbon and nitrogen compounds, the pathway of niacin synthesis is still obscure. Numerous possible precursors have been examined with preponderantly negative results. It has been noted by Euler *et al.* (1944) that the requirement of *Proteus vulgaris* for niacin is satisfied by the tetrahydro (guvacin) and hexahydro derivatives, but obviously synthesis need not proceed via these compounds. From an interesting study by Bonner and Beadle (1946) it was concluded that since each of three genetically-distinguishable *Neurospora* mutants was capable of effecting a different step in the formation of niacin at least as many intermediates were involved. One of these was

isolated and found to have the empirical formula $C_8H_9HNO_3$ (niacin- $C_8H_9NO_2$). Recent studies (Beadle *et al.*, 1947) have shown that *Neurospora* synthesizes niacin from tryptophan via kynurenine and hydroxyanthranilic acid (Beadle *et al.*, 1947; Bonner, 1948; Mitchell and Nye, 1948).

Of numerous organisms which have been surveyed, *Mycobacterium tuberculosis* appears to be the outstanding producer of niacin. Synthetic medium in which this organism was grown for 21 days was increased in content from 0.07 μ g. to 37 μ g./ml. in the cell-free liquor and to 61 μ g./ml. in the whole culture (Bird, 1947). Cultures of *Bacillus vulgatus* and *Bacillus mesentericus* also accumulate considerable niacin according to Burkholder and McVeigh (1942). Per gram of moist cells, *Bacillus vulgatus* produced 709 μ g. and *Bacillus mesentericus* 207 μ g., however, the concentrations reached were much lower than with *Mycobacterium tuberculosis*. Less striking, but definite evidence of synthesis by other bacteria as well as by *Oidium lactis*, *Penicillium camemberti* and unidentified yeasts have been described by Burkholder *et al.* (1943, 1944). Examination of niacin production by obligate anaerobes has been confined to *Clostridium butylicum* (Thompson, 1942). This organism elaborates appreciable amounts which are largely excreted into the medium.

Environmental factors and medium components which alter the degree of niacin synthesis have been recorded by several investigators, the full significance and explanation of which must await further study. Bakers' yeast which has been propagated aerobically in the presence of yeast extract (Van Lanen, 1946) or malt extract (Massock, 1943) is reduced in niacin potency. This depressing effect is unexpected since both of these materials are favorable to growth. It may be due to substances which limit synthesis, reduce the metabolic requirement, or enhance destruction of niacin. Lewis (1944) has found that the production of niacin by *Torula utilis* is increased by cultivation in the absence of iron. This is similar to observations with riboflavin (discussed elsewhere). However, the analogy with riboflavin is not complete since Eppright and Williams (1946) showed that the presence of KCN which has a favorable effect upon riboflavin production caused a reduction in niacin synthesis.

Of the factors which increase niacin synthesis, the finding that asparagin is stimulatory in synthetic medium (Van Lanen, 1946) is of special interest in view of the disclosure by Bovarnick (1943) that niacin or a compound with similar properties is formed by autoclaving asparagin in the presence of amino acids. Thiamine, likewise, improves the formation of niacin by *Aerobacter aerogenes* (Thompson, 1942) while

camphor and sulfaguanidine increase either its synthesis or retention (Eppright and Williams, 1946) by yeasts.

The importance of aerobic conditions for the elaboration of niacin by both yeasts and molds has been well established. Thompson (1942) found that *Aerobacter aerogenes* grown anaerobically produced only 41 percent of that obtained aerobically. Likewise, with bakers' yeast cultured in synthetic medium, aerobic cells were not only more than threefold richer in niacin but they excreted appreciably more vitamin into the medium (Van Lanen, 1946). The results of Singh *et al.* (1947) with various yeasts also suggest a correlation between the efficiency of aeration and niacin production.

3. Absorption from the Medium

Niacin, like thiamine, is readily absorbed from solution by yeasts, and probably by other microorganisms as well. Although of both practical and theoretical interest, it has not been determined how efficiently absorbed niacin is converted to the usual nucleotides. Under optimum conditions as much as 90% of the medium niacin may be concentrated in the cells (Massock, 1943; Van Lanen, 1946) and cell potencies of about 3 mg. of niacin/g. have been obtained (Massock, 1943). Such factors as (a) the composition of the medium, (b) availability of the vitamin, (c) conditions of cultivating the yeast, and (d) the particular species employed have been shown to alter the efficiency of absorption.

Contrasted with thiamine, absorption of niacin by yeast is dependent upon the presence of a fermentable substrate and excretion readily occurs following the utilization of the available carbohydrate (Van Lanen, 1946). Some forms of naturally-occurring niacin are not acquired by yeast despite their availability to such assay organisms as *Lactobacillus arabinosus*. Failure to permeate the cells may be responsible since it will be recalled that cocarboxylase is unavailable to some yeasts perhaps for this reason. Of the various types of yeast investigated, commercial bakers' yeast, and a distillers' grain yeast absorbed niacin most efficiently (Van Lanen, 1946). Because synthesis is favored by aerobic conditions whereas absorption occurs best anaerobically, enrichment of yeasts would be preferably accomplished by propagation aerobically followed by exposure to anaerobic conditions in the presence of fermentable substrate and available forms of the vitamin.

4. Niacin Inactivation

Harden and Young in their initial studies on codehydrogenase called attention to the lability of this coenzyme when it was present in yeast juice (1906). Subsequent studies with various organisms and tissues

indicate that the degradation of coenzymes I and II is common during active metabolism. Morel working with *Proteus vulgaris* (1941) found that the metabolic rate of this organism could be maintained only by the addition of nicotinamide. She attributed the utilization and need for this compound to a "wearing out" process which was a function of the number of oxidations and reductions undergone by the coenzyme. The inactivation of coenzyme in animal tissues results apparently from the splitting-off of nicotinamide, but Handler and Klein (1942) were unable to retard the degradation by supplementing with this compound.

From an examination of the niacin content of yeasts during aerobic and anaerobic propagation as well as from the analysis of various fermentation residues it may be seen that generally a net loss of niacin occurs, although in most instances the organisms employed are capable of its synthesis. Comparison of a single strain under aerobic and anaerobic conditions revealed that destruction was accelerated anaerobically (Van Lanen, 1946). Similarly, Swaminathan (1942) has observed that distillers' yeast is much lower in this vitamin than *Torula* or pressed yeast which was propagated aerobically. Since cells and culture liquors were analyzed following the liberation of bound niacin, it must be concluded that the metabolic breakdown of codehydrogenase proceeds further involving destruction of niacin itself. This destruction is probably correlated with accelerated codehydrogenase activity as occurs during fermentation. This utilization is distinguishable, quantitatively at least, from that described by Koser and Baird (1944) and Allinson (1943) in which niacin supplied both carbon and nitrogen for certain organisms. Nevertheless, it is readily apparent that by degradation the niacin level of microbial cells may be appreciably altered.

VI. BIOTIN

Biotin was first isolated from egg yolk in 1935 by Kögl and the isolation procedure was described in detail by Kögl and Tonnies in 1936. Later, Melville *et al.* (1942) and du Vigneaud *et al.* (1941) accomplished the isolation from milk and liver, respectively. The structure of biotin from liver has been established by du Vigneaud and coworkers; however, another structure, the existence of which has been questioned (Melville, 1944), has been proposed for the biotin from egg yolk. Hofmann (1945) and Melville (1944) have reviewed the chemistry and physiology of biotin.

Evidence recently accumulated indicates that biotin is linked in function with certain carboxylation, decarboxylation, and deamination reactions. The first insight into its biochemical role came from the observation that biotin, when present in relatively high concentration,

eliminated the requirement of *Lactobacilli* and *Streptococcus fecalis* for aspartic acid (Stokes *et al.*, 1947a and b). *L. arabinosus*, in addition, is capable of utilizing oxalacetic acid in the absence of both biotin and aspartic acid, in which case biotin-deficient cells are evidently capable of converting oxalacetic acid to aspartic acid by transamination (Landy, 1947). Similarly with *Escherichia coli*, failure to synthesize biotin is reflected in a reduced formation of α -ketoglutaric acid, the latter being involved, also, in aspartic acid production (Shive and Rogers, 1947). With biotin depleted cells, Lichstein and Umbreit (1947a) have demonstrated further that the decarboxylation of aspartic, malic, and oxalacetic acids and the deamination of aspartic acid, threonine, and serine (1947b) are retarded. These functions can be accelerated by the addition of biotin.

1. Biotin in Microorganisms

Studies on the quantitative distribution of biotin in microorganisms have not been extensive; available data are summarized in Table VI.

TABLE VI
Biotin Content of Cells and Whole Cultures

	Dry cells, $\mu\text{g./g.}$	Total synthesized, $\mu\text{g./g.}$ dry cells	References
Bacteria			
<i>Aerobacter aerogenes</i> (aerobic).....	3.9	47.9	315
<i>Aerobacter aerogenes</i> (anaerobic).....	2.4	13.4	315
<i>Azotobacter vinelandii</i>	2.56-4.23	149
<i>Clostridium butylicum</i>	1.7	1.8	315
<i>Proteus vulgaris</i>	3.4	24.1	315
<i>Pseudomonas fluorescens</i>	7.1	68.1	315
<i>Serratia marcescens</i>	4.1	34.1	315
Yeast			
<i>Candida arborea</i>	0.32	272
<i>Mycotorula lipolytica</i>	1.8	132
<i>Saccharomyces cerevisiae</i>	0.5-1.8	176, 272
<i>Torula utilis</i>	1.06-1.9	132, 157, 272
Fungi			
<i>Penicillium chrysogenum</i>	0.57-1.5	200, 301

Bacteria are particularly good sources of biotin with molds and yeasts being, in general, somewhat lower in potency. *Pseudomonas fluorescens* has been found to be one of the most productive with cells assaying 7.1 $\mu\text{g./g.}$ (Thompson, 1942). *Torula utilis* cultured in fruit juices

(Lewis *et al.*, 1944), molasses (Singh *et al.*, 1947), and wood sugar stillage (Kurth and Cheldelin, 1946) ranged from 1.0 to 1.9 $\mu\text{g./g.}$ *Saccharomyces cerevisiae* from molasses (Massock, 1943; Massock and Baldwin, 1943; Singh *et al.*, 1947) attained approximately the same potencies (0.5–1.5 $\mu\text{g./g.}$) while *Mycotorula lipolytica* and *Hansenula suaveolens* from wood sugars were slightly higher. On the other hand, *Candida arborea* produced significantly lower quantities than other yeasts (Singh *et al.*, 1947).

Among the molds, the mycelium of *Penicillium chrysogenum* grown on corn steep-lactose medium showed about the same level of biotin as the yeasts (0.57 to 1.5 $\mu\text{g./g.}$).

Various bacteria cultured on biotin-free media elaborate biotin (Landy and Dicken, 1941; McIntire *et al.*, 1941; Wright and Skeggs, 1944). Culture liquors from such organisms have been observed to range from 0.012 to 0.05 $\mu\text{g./ml.}$, but substantially greater synthesis has been effected during the fermentation of sugar to fumaric acid by a *Rhizopus* species. In one instance, 1 g. biotin (equivalent to 0.13 $\mu\text{g./ml.}$) was recovered from 2000 gallons of fermented medium (Schivek, 1944).

Avidin, a protein found in raw egg white which forms a stable complex with biotin and certain biotin analogues (Eakin and Williams, 1940; du Vigneaud *et al.*, 1942b) inhibits the growth of those species which require an exogenous biotin supply, but has not been shown to enhance biotin production by those capable of its synthesis.

2. Absorption by Microbial Cells

During a survey of the vitamin content of commercial yeasts, Massock (1943) observed an appreciable variation in biotin content. The possibility of absorption from the medium was investigated as an explanation with the finding that as much as 90% of the biotin present in the medium could be removed by the cells. Consequently, cane molasses which contains from five to ten times as much biotin as beet molasses gives rise to cells of higher potency.

Bacteria which are heterotrophic for biotin absorb it in amounts which do not greatly exceed their metabolic requirements and inactivate that added in excess (Krueger and Peterson, 1948; Thompson, 1942). Of the total produced by bacteria capable of synthesis, amounts ranging from 10 to 98% have been found in the medium (Landy and Dicken 1941; McIntire *et al.*, 1941; Thompson, 1942). Therefore, compared with yeasts, bacteria have a lower excretion threshold with respect to this vitamin.

3. Biotin Analogues

Oxybiotin (*O*-heterobiotin), an analogue of biotin in which the sulfur is replaced by oxygen, has been synthesized (Duschinsky *et al.*, 1945; Hofmann, 1943, 1945) and its ability to promote the growth of various

organisms requiring biotin has been followed by several investigators (Duschinsky *et al.*, 1945; Krueger and Peterson 1948; Pilgrim *et al.*, 1945; Rubin *et al.*, 1945; Ryan *et al.*, 1945). These studies have revealed that *dl*-oxybiotin compared with *d*-biotin is 50% as active for *Lactobacillus arabinosus* and *L. pentosus*, 40% as active for *L. casei*, and only from 1-25 % as active for *Saccharomyces cerevisiae* and *Rhizobium trifolii*. Only *d*-biotin is biologically active (Stokes and Gunness, 1945, and if true for oxybiotin it has essentially equivalent activity for the two *Lactobacilli* named above but lower activity for the other organisms.

Whether the oxygen analogue is utilized per se or is converted to biotin has been a subject of considerable controversy (Hofmann and Winnick, 1945; Rubin *et al.*, 1945). Based upon differential assays following treatment of preparations to eliminate either biotin or oxybiotin, impressive evidence has been offered that oxybiotin is not converted to biotin by yeast (Axelrod *et al.*, 1946; 1947; Hofmann *et al.*, 1945, 1947) or by lactobacilli (Krueger and Peterson, 1948).

Desthiobiotin, which is obtained by cleaving the thiophene ring and replacing the sulfur with two hydrogen atoms, is the only biotin analogue possessing yeast growth activity comparable to biotin (Lilly and Leonian, 1944; Melville *et al.*, 1943). In contrast to oxybiotin, desthiobiotin when utilized is converted to biotin (Dittmer *et al.*, 1944; Leonian and Lilly, 1945). With *Escherichia coli* this reaction is prevented by 2-oxo-4-imidazolidinecaproic acid. Since an x-ray mutant of *Penicillium chrysogenum* synthesized desthiobiotin but was incapable of completing the synthesis to biotin, Tatum has suggested that the former may be a normal intermediate in biotin formation (1944, 1945). With the same organism, it was demonstrated that pimelic acid stimulated desthiobiotin production (Tatum, 1945) apparently giving rise to the valeric acid side-chain. Early reports that this compound stimulated biotin synthesis by diphtheria bacilli (du Vigneaud, 1942a) and *Aspergillus niger* (Eakin and Eakin, 1942) were thus confirmed. However, *Ashbya gossypii*, *Neurospora*, *Ceretostomella* and certain *Fusarium* species are unable to utilize pimelic acid to produce biotin (Robbins and Ma, 1942).

Seven 1,3-disubstituted ureas which may be looked upon as open chain analogues of desthiobiotin were biologically inactive for both *Lactobacillus casei* and *L. arabinosus* (Schultz, 1947).

VII. *p*-AMINOBENZOIC ACID

1. Production by Microorganisms

Recent reviews (Ansbacher, 1944; Sevag, 1946) have been devoted wholly or in part to considerations of the role of *p*-aminobenzoic acid (PABA) in the nutrition of animals and microorganisms. While the

status of PABA per se as a member of the B vitamin group has been questioned (Hogan and Kamm, 1943; Sevag *et al.*, 1946) the evidence which has accrued in its favor is impressive. Analogous with the established members of the water-soluble group, it is widely distributed in nature and is either synthesized by microorganisms or required for their growth. That PABA might be an essential nutrilit for fungi was predicted first by Woods (1940) following the demonstration of its ability to overcome inhibition by sulfonamide. This hypothesis was confirmed by Rubbo and Gillespie (1940) with the finding that PABA was required for the growth of *Clostridium acetobutylicum* and by its actual isolation from yeast (Blanchard, 1941). The number of species which have been found to require an external source of PABA now has grown to about fifteen (Woods, 1947).

Whether the essentiality of PABA resides solely in its being a constituent of the folic acid molecule remains to be ascertained; several observations which have been summarized by Woods (1947) lead away from this conclusion. Based upon (a) the additional requirements of certain organisms when grown in the presence of sulfa compounds, and (b) the decreased need for PABA in the presence of other substances, notably methionine and adenine, it is believed that the function of PABA also is associated with the formation of amino acids and purine bases (Lampen *et al.*, 1946, 1947; Woods, 1947).

PABA is produced by bacteria (Eppricht and Williams, 1946; Landy *et al.*, 1943b) yeasts (Lampen *et al.*, 1945; Lewis, 1944), and molds (Hartelius, 1946) cultivated on various synthetic substrates from which the factor has been omitted. Landy *et al.* (1943b) showed that of thirty widely different organisms, PABA appeared in measurable amounts in all cultures capable of initiating growth in the basal media. The quantities formed ranged from 0.003 to 0.241 $\mu\text{g./ml.}$ Similar concentrations have been found in culture liquors of *Mycobacterium tuberculosis* (Eppricht and Williams, 1946). In both of these reports it is emphasized that the majority of the PABA of bacterial cultures was located outside the cells.

Although variations between and within species are prominent, yeasts (Table VII) are exceptionally potent in this substance and are probably the richest natural source of PABA. Of the various types examined, commercial bakers' and brewers' strains are comparatively high while the species grown on wood sugar stillage are low.

Since the better synthetic media which have been employed result in yields of the same order of magnitude as molasses and other natural substrates (Lampen *et al.*, 1945), it appears that neither precursors nor absorption are major factors in determining cell potencies. In fact

TABLE VII
p-Aminobenzoic Acid Content of Yeasts

	Dry cells, μg./g.	References
<i>Hansenula suaveolens</i>	16	132
<i>Mycotorula lipolytica</i>	31	132
<i>Saccharomyces cerevisiae</i>	7.8-95	133
<i>Torula utilis</i>	17-21	132, 157
Commercial foil yeast.....	28-53	133
Commercial bakers' yeast.....	22-175	133
Commercial brewers' yeast.....	9.3-102	138, 155

much of the PABA synthesized by yeast is released into the medium. Lampen *et al.* (1945) and Lewis *et al.* (1944) found from about 70 to 98% of that fabricated was excreted, the cell-free liquors in the former study increasing from 0.25 to 6.2 μg./ml.

Relatively few molds have been examined for their ability to produce PABA, but positive evidence has been secured with *Aspergillus niger* (Hartelius, 1946) and with most strains of *Neurospora* (Tatum and Beadle, 1942).

In contrast to many natural products, PABA exists in yeast cells predominantly in a water-extractable form (Lampen *et al.*, 1945; Lewis, 1942). The small amount of bound PABA which can be demonstrated may comprise that in folic acid and in such similar peptides as described by Ratner *et al.* (1946).

2. Factors Influencing PABA Synthesis

The inhibition of growth by sulfonamide has been ascribed to its interference with metabolic reactions involving PABA. If true, with those organisms capable of PABA synthesis, accelerated activity tending to overcome the inhibition might be anticipated. On this point, however, experimental data are contradictory. With yeast (Landy and Dicken, 1942a), *gonococci* (Landy and Gerstung, 1945) and *Staphylococcus aureus* (Landy *et al.*, 1943a; Spink *et al.*, 1944) acclimatization to sulfa compounds is accompanied by the increased production of PABA. Nevertheless, Lemberg *et al.* (1946) following similar procedures were unable to demonstrate any greater synthesis by *Escherichia coli* although an arylamine, largely anthranilic acid, appeared earlier in cultures containing sulfathiazole. Sevag and Green (1944) also present evidence to show that PABA formation is not directly related to sulfonamide resistance.

Iron may be required for the synthesis of PABA according to Lewis (1944) since production by *Torula utilis* decreased from 60 to only 3

μg./g. when the medium lacked iron. Eppright and Williams (1946) found that fluoride but not cyanide effectively retarded PABA formation by *Saccharomyces cerevisiae*.

VIII. PYRIDOXINE

The studies of Snell *et al.* in 1942 indicated that pyridoxine which had been synthesized in 1939 (Stiller *et al.*) was only one of a group of naturally-occurring compounds possessing vitamin B₆ activity. This conclusion was reached after it was observed that the pyridoxine content of various natural materials was insufficient to account for their growth-promoting activity for certain lactic acid bacteria. The unusually high response of such substances, tentatively termed "pseudopyridoxine" was shown subsequently to be due to pyridoxal and pyridoxamine which are formed, respectively, through the oxidation and amination of pyridoxine (Harris *et al.*, 1944; Snell, 1944b). Based upon the differential response of *Saccharomyces cerevisiae*, *Lactobacillus casei*, and *Streptococcus fecalis* to these compounds their measurement in various products is now possible (Snell, 1945). Further studies recently conducted with both microorganisms and animals have resulted in the disclosure that pyridoxal phosphate is the coenzyme in transamination (Lichstein *et al.*, 1945; Schlenk and Snell, 1945), decarboxylation (Lichstein *et al.*, 1945), and possibly carboxylation (Lyman *et al.*, 1946) reactions.

Microorganisms, as may be seen from the data in Table VIII, are appreciably better sources of pyridoxine than higher plant and animal products. Yeasts are particularly rich with brewers' yeast somewhat superior to bakers' yeast. Provided that the slightly greater potency of anaerobically propagated yeast is reproducible, it may be attributable to a greater absorption under anaerobic conditions. Lewis *et al.* (1944) have reported that *Torula utilis* absorbs some pyridoxine from the medium and similar observations have been made with lactic acid bacteria (Bohonos *et al.*, 1942). Another contributing factor to cell potencies is apparent from the data of Thompson (1942) who found that while the total synthesis of pyridoxine by *Aerobacter aerogenes* was nearly equivalent under aerobic and anaerobic conditions, less excretion took place anaerobically.

Microorganisms appear to contain all of the forms of vitamin B₆ which have thus far been described. Snell concluded from assays of yeast extract that pyridoxamine was the predominant form there, although pyridoxal was present (Snell, 1945). *Neurospora*, likewise, contains both pyridoxine and "pseudopyridoxine" (Stokes *et al.*, 1943). Pyridoxal phosphate is much less active to the assay organisms than the other members of the group but becomes active following acid hydrolysis

TABLE VIII
Pyridoxine Content of Microorganisms and Culture Liquors

	Dry cells, μg./g.	Total synthe- sized in cells and medium, μg./g. dry cells	References
Bacteria			
<i>Aerobacter aerogenes</i> (aerobic).....	6.8	26.8	315
<i>Aerobacter aerogenes</i> (anaerobic)....	18	18.23	315
<i>Serratia marcescens</i>	11	34	315
<i>Pseudomonas fluorescens</i>	5.7	75.7	315
<i>Proteus vulgaris</i>	6.8	16.4	315
<i>Clostridium butylicum</i>	6.2	23.2	315
Yeasts			
<i>Torula utilis</i>	35	157
Commercial bakers' yeast.....	16 ¹ -65	15, 27, 224, 245
Commercial brewers' yeast.....	25-100	15, 27, 172, 245, 270, 292
Molds			
<i>Neurospora sitophila</i>	9.1-13.7	293
<i>Penicillium chrysogenum</i>	23	301

¹ Calculated to a dry substance basis assuming 30 % initial solids.

and apparently constitutes a substantial portion of the bound pyridoxin of yeast and other natural products (Rabinowitz and Snell, 1947). The existence of still other forms, one labile to acid and the other requiring more rigorous hydrolysis procedures (Melnick *et al.*, 1945) also have been hypothesized.

The formation of pyridoxine and pyridoxal phosphate is accomplished by various organisms cultivated on synthetic media free of the vitamin B₆ group (Bellamy *et al.*, 1945; Lewis, 1944; Stokes *et al.*, 1943). Although the mechanism involved in the synthesis of pyridoxine, pyridoxal, and pyridoxamine has not been elucidated, it has been demonstrated that growing cultures of organisms which are capable of utilizing these compounds convert them to the coenzyme. In addition, resting cells of *Streptococcus fecalis* convert pyridoxamine to codecarboxylase when provided with pyruvate (Bellamy *et al.*, 1945) as receptor for the amino group.

Little attention has been given to factors affecting the degree of pyridoxine synthesis by microorganisms. Snell, and Snell and Guirard (1944a, 1943) reported that the requirement of *S. fecalis* for pyridoxine is diminished by supplementing the medium with alanine. One explana-

tion proposed for this specific effect of alanine is that it is a precursor of pyridoxine (Snell and Guirard, 1943). Opposed to this theory, however, are the facts that (a) the basal media upon which the effect is demonstrable contain some alanine (Snell, 1944a) and (b) codecarboxylase apparently is not formed when alanine is substituted for pyridoxine (Bellamy *et al.*, 1945).

With a pyridoxine-less mutant of *Neurospora*, Stokes *et al.* (1943) reported that pyridoxine, while not produced under the usual conditions, was formed when the medium was adjusted to pH 5.8 or above and ammonium salts were supplied. Since pyridoxine was shown to be present in these *Neurospora* cells, it suggests that the biochemical defect in such cultures is circumvented by the selection of culture conditions which either permit a particular reaction to function or obviate its necessity. With *Torula utilis* propagated on synthetic medium to which varying levels of iron were added, it was found that maximum synthesis of pyridoxine occurred when the concentration of iron was 0.1 p.p.m. which was suboptimal for growth (Lewis, 1944). Specific inhibitors for enzymes involved in fermentation did not retard the formation of pyridoxine by *Saccharomyces cerevisiae* (Eppright and Williams, 1946), indicating that its function in metabolism may be confined to the reactions discussed above. The pyridoxine content of cells grown on galactose was unusually high whereas on xylose little or none was synthesized (Eppright and Williams, 1946).

IX. INOSITOL

Although *m*-inositol has been known for many years, its biological importance was unrecognized until 1928 when Eastcott isolated and identified it as a member of Wildiers's "bios." Despite this early and auspicious linkage with the water-soluble group and more recent confirmation of vitamin activity, the occurrence and quantitative production of inositol by microorganisms has received little attention. The lack of suitable analytical methods, recently obviated by the development of microbiological procedures, has been one of the main reasons for the paucity of data on the distribution of inositol.

In nature, inositol occurs in stable complexes such as the lipositols and phytin which are resistant to the commonly-employed hydrolytic procedures. This may explain in part the wide variations which have been noted in the content of bacteria and yeasts (Table IX). Because of the difficulty in liberating inositol, the values given especially for bacteria wherein enzyme digestion was used should be considered minimal.

TABLE IX
Inositol Content of Microorganisms

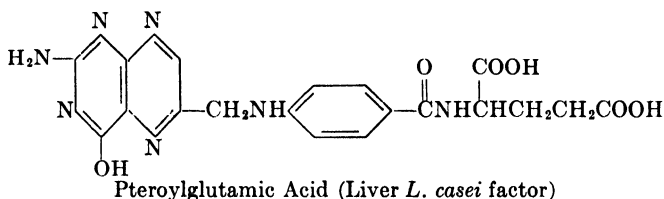
	Dry cells, μg./g.	References
Bacteria		
<i>Aerobacter aerogenes</i> (aerobic).....	1400	315
<i>Aerobacter aerogenes</i> (anaerobic).....	1600	315
<i>Serratia marcescens</i>	1600	315
<i>Pseudomonas fluorescens</i>	1700	315
<i>Proteus vulgaris</i>	1000	315
<i>Clostridium butylicum</i>	870	315
Yeasts		
<i>Torula utilis</i>	3400-3600	157
Brewers' yeast.....	2700-5000	157, 347

The limited data do not permit drawing conclusions regarding factors influencing the inositol content of microorganisms. Apparently some absorption is accomplished by yeast since Eastcott (1928) remarked that culturing in a medium devoid of inositol reduced the content to one-half that from an inositol-rich medium. According to Lewis (1944) an iron deficiency decreases by 25 to 50% the inositol synthesis by *Torula utilis*. *T. utilis* from a sucrose-inorganic salts medium contained from 600 to 2600 μg. inositol/g. dry cells depending upon the amount of iron supplied. When cultured in various fruit juices, the same species contained 2500-2850 μg./g. (Lewis *et al.*, 1944). That these values may be low is suggested by assays performed later employing more vigorous hydrolysis. Beadle and Horowitz using the *Neurospora* assay and refluxing the samples for 2 hours in 3% sulfuric acid obtained values of 3400 and 3600 μg./g. respectively, for yeasts propagated on molasses and prune juice (Lewis *et al.*, 1947). A sample of brewers' yeast which assayed 2700 μg./g. following mild hydrolysis was found to contain 4800 μg./g. after refluxing for 6 hours in 18% HCl (Lewis *et al.*, 1947). Woolley's values (1941) of 5000 μg./g. for brewers' yeast are in good agreement.

X. FOLIC ACID

Earlier reviews have dealt specifically with the various factors belonging to the folic acid group (Piffner and Hogan, 1946), and generally with vitamin deficiencies following the administration of sulfonamides (Daft and Sebrell, 1945). These should be consulted to provide a background for the present discussion. Much of the confusion in evidence at the time of these publications relative to the chemical nature, biological properties, and nomenclature of the various substances pos-

sessing folic acid activity has since been resolved with the determination of structure and synthesis of the liver *Lactobacillus casei* factor (pteroylglutamic acid) (Angier *et al.*, 1945).



In yeast and a fermentation product conjugated forms of pteroylglutamic acid occur which have been identified respectively, as pteroylheptaglutamic acid (Pfiffner *et al.*, 1946, 1947) and pteroyltriglutamic acid (Hutchings *et al.*, 1946b). Aside from these, evidence has accumulated which points toward the existence in products of microbiological origin of still other conjugates or biologically active compounds related to pteroylglutamic acid (Keresztesy *et al.*, 1943; Mills *et al.*, 1944; Ratner *et al.*, 1946).

1. Content of Microorganisms

Shown in Table X are the folic acid potencies of various microorganisms. Considerable attention has been given to yeasts because of their commercial availability and relatively high content, although several bacteria and lower fungi also have been examined. Conspicuous are the high potencies of certain species with respect to this vitamin and, compared with other B vitamins, a more pronounced variation in content. It should be mentioned that some of the variation is more apparent than real and results undoubtedly from the failure to completely liberate bound folic acid. This is especially true of the values given for bacteria since this investigation (Thompson, 1942) was carried out prior to the development of improved extraction procedures. While some organisms are low in folic acid content all but a few are capable of its synthesis and in only one recorded instance, that in which lactic acid bacteria were cultivated with thymine replacing folic acid (Stokes, 1944) have bacteria devoid of this substance been obtained.

Among the yeasts *Saccharomyces* species grown under both commercial and experimental conditions and *Candida arborea* are excellent sources of folic acid. Inherent differences in synthetic ability between species are indicated in the data of Agarwal *et al.* (1947). They obtained the following average values for *Saccharomyces cerevisiae*, *Candida arborea*, *Torula utilis*, and *Oidium lactis* when each was grown on four different molasses media: 20.4, 15.9, 12.5, and 7.2 $\mu\text{g./g.}$ respectively.

TABLE X
Folic Acid Content of Microorganisms

	Dry cells μg./g.	References
Bacteria		
<i>Aerobacter aerogenes</i> (aerobic).....	2.8 ¹	315
<i>Aerobacter aerogenes</i> (anaerobic).....	1.0 ¹	315
<i>Clostridium butylicum</i>	0.5 ¹	315
<i>Proteus vulgaris</i>	4.2 ¹	315
<i>Pseudomonas fluorescens</i>	1.8 ¹	315
<i>Serratia marcescens</i>	3.2 ¹	315
Yeasts		
<i>Candida arborea</i>	14.8-19.6	3, 272
<i>Hansenula suaveolens</i>	1.7	132
<i>Mycotorula lipolytica</i>	3.1	132
<i>Oidium lactis</i>	5.6-14.8	3, 272
<i>Saccharomyces cerevisiae</i>	19.1-35.6	3, 272
<i>Torula utilis</i>	3.8-31.2	3, 132, 272
Commercial yeasts.....	15-80	162
Molds		
<i>Penicillium chrysogenum</i>	13.4-14.6	200
<i>Penicillium notatum</i>	3.6-4.5	200

¹ Values calculated assuming that the standard employed was folic acid of 20 % purity.

Environmental factors affecting folic acid formation have received little attention. Since approximately equivalent yields have been secured with *Torula utilis* grown on synthetic (Lewis, 1944) and molasses media (Agarwal *et al.*, 1947) it is probable that absorption from the medium is not an important factor in determining cell contents. Nevertheless, more than fourfold variations in commercial yeast preparations were noted (Lillie and Briggs, 1947) which suggests the existence of pre-determining factors. Singh *et al.* (1947) have found in most instances that yeasts were decreased in folic acid content when the rate of aeration was favorable to high yields of cells. Supplementing the medium with iron (Lewis, 1944) or sulfite (Epprawright and Williams, 1946) stimulated, whereas cultivation in saturated camphor or with galactose as the source of carbon lowered (Epprawright and Williams, 1946) the synthesis of folic acid by yeasts. Three yeasts, *Mycotorula lipolytica*, *Torula utilis*, and *Hansenula suaveolens* grown on wood sugar stillage were abnormally low in content (Kurth and Cheldelin, 1946).

Whereas with yeasts, high aeration rates lower folic acid potencies, anaerobic conditions retard production by bacteria. *Clostridium butylicum* and anaerobically-cultured *Aerobacter aerogenes* were significantly lower in folic acid than *A. aerogenes* and other bacteria grown

aerobically. A large part of the vitamin elaborated by *A. aerogenes* under aerobic conditions was found in the medium (Thompson, 1942). It might be construed from this that synthesis by bacteria is enhanced by aerobic growth while the metabolic requirement is not increased, resulting in excretion.

2. Biosynthesis of Folic Acid

An extensive survey of microorganisms for their ability to synthesize folic acid was conducted by Burkholder *et al.* (1945). Bacteria were cultivated on a medium composed of casein with supplementary amino acids, dextrose, salts, and vitamins other than folic acid. For the yeasts and molds the medium contained asparagin, glucose, salts and vitamins exclusive of folic acid. All cultures were aerated by shaking. Folic acid was liberated prior to assay by subjecting the whole cultures to the action of conjugase from chick pancreas.

Group	No. tested	Folic acid produced, μg./ml.
Bacteria	42	0.03-0.69
Yeasts	369	0.01-0.36
Lower fungi	84	0.002-0.096

From these data it would appear that bacteria are superior to yeasts and lower fungi as producers of folic acid; however, yeast cells (Table X) are generally richer in folic acid than bacteria. Presumably yeasts tend to retain the vitamin while most bacteria excrete it. From the results of Thompson *et al.* (1942) it is seen that from 50 to 88% of the total folic acid produced was located in the medium. On the other hand the SLR factor which can replace folic acid for *Streptococcus fecalis* is stored almost completely in the cells of enterococci (Stokes and Larsen, 1945).

Conjugase treatment applied to bacterial cultures does not result in a large release of folic acid in a manner corresponding to the treatment of yeasts and yeast extract (Burkholder *et al.*, 1945). In general bacterial folic acids, therefore, may not be conjugated, or they may be conjugated differently than in *Corynebacterium* (see below).

Data given by Burkholder *et al.* for synthesis by *Aerobacter aerogenes* are substantially higher than that found by Wright and Skeggs (1944) for the same organisms but are similar to those for *Clostridium acidi urici* (Barker and Peterson, 1944), *Escherichia coli* (Miller, 1944) and *Mycobacterium tuberculosis* (Bird, 1947). Cultures of the latter three organisms ranged from 0.024 to 0.05 μg. folic acid/ml. However, in an undefined medium fermented with a strain of *Corynebacterium*, synthesis about one hundred fold greater than the average for bacteria (Burkholder

et al., 1945) has been secured (Hutchings *et al.*, 1946a). This compound termed "fermentation folic acid" and shown to be pteroyltriglutamic acid has activity comparable to pteroylglutamic acid for *L. casei*, chicks (Jukes and Stokstad, 1947) and probably other animals (Spies, 1946; Totter, 1946).

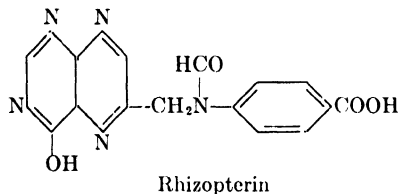
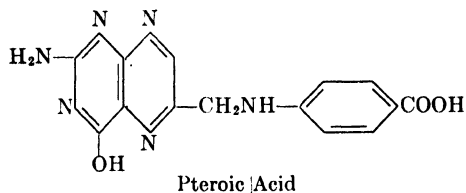
3. Production from Precursors

It has been shown by Burkholder *et al.* (1945) that certain bacteria are capable, not only of synthesizing folic acid, but also of releasing it from bound form in yeast and yeast extract. Such liberation requires according to Pffner *et al.* (1946) a carboxypeptidase which is found rather widely distributed among bacteria and markedly active in *Bacillus subtilis*, *Bacillus vulgatus*, *Serratia marcescens*, and an unidentified organism isolated from the intestines of a fowl. When grown on a medium containing 2% yeast extract, and 4% dextrose and having initially about 0.06 $\mu\text{g.}$ free and 1.04 $\mu\text{g.}$ total vitamin/ml., these organisms increased the free folic acid concentration to 0.48 to 1.67 $\mu\text{g./ml.}$ An incubation period of 3 days was optimum for the conversion (Burkholder *et al.*, 1945).

In the case of other B vitamins, *e.g.*, thiamine or pantothenic acid, it has been shown that certain microorganisms are limited in their ability to fabricate the molecule until provided with certain assimilable fragments thereof. A similar situation is indicated with folic acid from the limited data that have been compiled with intermediates thus far available. Mills *et al.* (1944) found when *Mycobacterium tuberculosis* was propagated on a synthetic medium to which *p*-aminobenzoic acid was added, chick growth factors of the folic acid type (vitamins B₁₀ and B₁₁) were increased in yield. These results are readily explainable by the incorporation of *p*-aminobenzoic acid into these compounds, probably in this instance conjugates having folic acid activity. Likewise, Sarrett (1947) has observed that *Lactobacillus arabinosus* but not *L. casei* can effect the formation of folic acid when supplied with an adequate amount of *p*-aminobenzoic acid. From 5 to 15% of the PABA added was recovered as folic acid following a 70-hour incubation period.

The production of pteroylglutamic acid by microorganisms given an exogenous supply of *p*-aminobenzoyl glutamic acid, the appropriate pterin or pterioic acid should contribute toward unfolding the mechanism of biosynthesis. Pterioic acid (molecule lacking glutamic acid) has been found active in promoting growth of *S. fecalis* but not *L. casei* (Waller *et al.*, 1946). In this respect as well as structurally it resembles the SLR factor first described by Keresztesy *et al.* (1943) and Stokes and Larsen (1945). This factor which is synthesized by *Rhizopus nigricans* and

which has been characterized and termed rhizopterin by the Merck group (Wolf *et al.*, 1947) may be looked upon as a formylated pteronic acid.



While the above are utilized by certain organisms, leucopterin and isoxanthopterin have been found inactive for *Aerobacter aerogenes* (Wright and Skeggs, 1944) and xanthopterin, convertible to folic acid by surviving rat liver (Wright *et al.*, 1944), actually inhibits folic acid elaboration by this organism.

Sulfa compounds were detected by Miller (1944) to retard the production of folic acid. According to Lampen and coworkers this is due to the interference by such compounds with the incorporation of *p*-aminobenzoic acid into pteroylglutamic acid (1946, 1947).

XI. ASCORBIC ACID

Although the presence of ascorbic acid in fungi would not be unexpected in view of its wide distribution and abundance among higher plants, little conclusive evidence has been published until recently. By staining with silver nitrate, the specificity of which is questionable, the occurrence of this vitamin in numerous microorganisms has been indicated (Bourne and Allen, 1935). Confirmation of its presence, however, through animal assay or isolation, for the most part, has been lacking. While too early to draw any conclusions regarding its universal presence in microorganisms, it has been established definitely that some are capable of its synthesis.

By determining reducing activity Lewis (1939) investigated the production of ascorbic acid by several molds cultured on potato medium and Knop's solution. He observed that *Aspergillus niger*, *Cephalothecium roseum*, and *Poecilomyces* sp. increased the reducing activity of the potato substrate two- to threefold during an incubation period of 22 days. In Knop's solution after 17 days' incubation, wet mycelia

of *Aspergillus niger*, *Aspergillus flavipes*, *Cephalothecium roseum* and *Penicillium ramosus* contained respectively 1.38, 0.72, 1.39 and 0.34 mg./g. of reducing substances calculated as ascorbic acid. Most of the vitamin on the latter medium was intracellular although a small quantity appeared in the cell-free liquor.

Various *Aspergilli*, *Penicillia*, and *Mucor* species were investigated by Cuffaro (1940) who secured positive evidence only with *Mucor rosea*. The maximum yield which accumulated in the sorbose-nutrient salts medium was equivalent in reducing activity to 0.72% ascorbic acid. Gottlieb and Gilligan (1946) were unable to demonstrate the presence of ascorbic acid in mycelia of *Alternaria*, *Thielavia*, *Rhizoctonia*, *Helminthosporium*, and *Fusarium*.

By means of titration with 2,6-dichlorophenolindophenol Bernhauer *et al.* (1936) followed the formation of ascorbic acid by *Aspergillus niger* grown under various conditions. Highest yields (up to 13.9 mg. per 100 ml. of medium) were obtained with sucrose, sorbitol, glycerol, and inositol and were correlated with the concentration of carbohydrate employed. These observations with regard to *Aspergillus niger* were confirmed and extended by Geiger-Huber and Galli (1945). The reducing substance produced was identified with ascorbic acid both by preparation of the 2,4-dinitrophenylhydrazine derivative and by animal assay. Their culture liquors contained from 1 to 5 mg./100 ml.

With *Aspergillus niger* (Bernhauer *et al.*, 1936; Galli, 1946; Geiger-Huber and Galli, 1945) and *Mucor rosea* (Cuffaro, 1940) there is general agreement that relatively long incubation periods (6–22 days), certain minerals, high sugar concentrations, a pH of 2.0 to 3.8, adequate nitrogen source and a slightly elevated temperature are conducive to ascorbic acid production. Ketogulonic acid may be an intermediate in this fermentation (Galli 1946).

Few bacteria have been examined for their ability to elaborate ascorbic acid. Busing and Peters (1940), however, have shown that it is formed by *Bacillus prodigiosus* when grown on xylose. During a 12-day incubation period the reducing activity of the medium, as ascorbic acid, increased about 13 mg. %. Animal feeding trials substantiated the conclusion that at least a portion of the substance produced was active biologically.

Although yeasts can absorb ascorbic acid from extracts of natural products (Trufanov and Ennatskaya, 1944), they are of low potency (Boas-Fixen and Roscoe, 1940) and probably cannot fabricate the molecule. However, Lunde and Lie (1938) found marine algae to be a fairly good source of ascorbic acid, ranging from 5 to 140 mg./100 g. wet substance.

XII. ERGOSTEROL

Ergosterol, long known to be a component of the lipid fraction of microorganisms, was first isolated by Tanret (1889, 1890) from ergot. Gerard (1892, 1895a and b, 1898) at about the same time, found it widely distributed among fungi. With the discovery that, upon irradiation, ergosterol was converted to an antirachitic substance (vitamin D₂, calciferol) this compound assumed increasing importance. The physiology and biochemistry of ergosterol has been discussed at some length by Bills (1935).

In microorganisms, ergosterol occurs principally in bound form. The palmitate ester has been isolated from several strains of *Penicillia* (Oxford and Raistrick, 1933) and more recently from *Aspergillus fumigatus* (Weiland and Prelog, 1947). Characterization of the bound form from yeast, although attempted, has been unsuccessful (Maguigan and Walker, 1940).

1. Ergosterol in Bacteria

The quantitative distribution of ergosterol in bacteria and other microorganisms is summarized in Table XI. Few bacteria have been examined quantitatively. *Mycobacterium tuberculosis*, an organism distinctive for its high lipid content, reported to be devoid of this provitamin (Anderson 1932; Anderson *et al.*, 1931; Prickett *et al.*, 1930) was later found to contain it (Hecht, 1935). On the other hand, *Bacillus butyricus* has been found to contain 0.49% ergosterol.

2. Ergosterol in Molds

Numerous molds have been investigated and almost without exception they have been shown to contain ergosterol. While molds have not been considered an important commercial source of the provitamin, industrial utilization in the form of by-product mycelia from some of the recently-developed mold fermentations would not be unexpected. For example, Zook *et al.* (1944) isolated ergosterol in crystalline form equivalent to 1% of the dry, surface-grown mycelium of *Penicillium notatum*. Cavallito (1944) confirmed the above observation with regard to surface cultures of this organism, but noted only traces in the mycelium of submerged cultures. Some strains of *P. notatum* are productive under submerged growth conditions, however, since Savard and Grant (1946) found 0.22% ergosterol in commercially-propagated mycelium from strain X-1612.

From a survey of numerous molds employing surface growth, ergosterol synthesis was found to be governed by the particular strain as well as by the cultural conditions. With *Aspergillus fischeri* (Wenck *et al.*,

TABLE XI
Ergosterol Content of Microorganisms

	Dry cells, %	References
Bacteria		
<i>Bacillus butyricus</i>	0.49	101
Yeasts		
<i>Blastomyces dermatitidis</i>	0.24	214
<i>Endomyces hordie</i>	0.2	26
<i>Endomyces fibuliger</i>	0.2	26
<i>Endomyces lindneri</i>	0.2	26
<i>Monilia albicans</i>	0.4	214
<i>Mycoderma</i> sp.....	0.2	26
<i>Nadsonia fulvescens</i>	0.3	26
<i>Saccharomyces carlsbergensis</i>	2.0	26
<i>Saccharomyces pastorianus</i>	0.3	26
<i>Torula pulcherrima</i>	0.2	101
<i>Torula utilis</i>	0.5	199
<i>Zygosaccharomyces chevalieri</i>	0.2	26
<i>Zygosaccharomyces mandshuricus</i>	0.2	26
<i>Zygosaccharomyces mellaleri</i>	0.54	101
Commercial debittered brewers' yeast.....	0.18-0.34	199
Commercial brewers' strain (aerobic).....	1.4	26
Commercial primary grown yeast.....	0.3-0.8	26, 199
Commercial distillers' strain.....	0.3	26
Commercial pressed yeast.....	0.4-2.7	87, 88
Molds		
<i>Penicillium aurantio-bruneum</i>	0.18	130
<i>Penicillium aurantio-griseum</i>	0.5	209
<i>Penicillium biourgeanum</i>	0.02	209
<i>Penicillium brevi-compactum</i>	0.5	209
<i>Penicillium chrysogenum</i>	1.0-1.1	48
<i>Penicillium citrinum</i>	1.1-1.3	48
<i>Penicillium expansum</i>	0.14	221
<i>Penicillium glaucum</i>	0.75	101
<i>Penicillium grisio-bruneum</i>	0.04	209
<i>Penicillium notatum</i> (surface growth).....	1.0-1.1	48, 359
<i>Penicillium notatum</i> (submerged growth).....	0.22	360
<i>Penicillium puberulum</i>	0.13	29
<i>Penicillium zanthinellum</i>	0.06	221
<i>Pullularis</i> (<i>Dematium</i>) <i>pullulans</i>	0.29	101
<i>Aspergillus fischeri</i>	2.23	332
<i>Aspergillus niger</i>	0.14	101
<i>Aspergillus oryzae</i>	0.26	221

1935) the potency increased with the carbohydrate supply. The highest yield, 2.23 %, was secured in a medium composed of 20 % glucose, 0.5 % urea, and 0.68 % potassium acid phosphate. The nitrogen source also

was a factor, urea being most suitable when calcium carbonate was present and nitrate when it was omitted.

3. *Synthesis by Yeasts*

Despite the fact that yeast has been one of the principal sources of provitamin D for many years, the various factors affecting its synthesis have not been clearly defined. As with the molds both strain variations and culture conditions have been implicated. At one time, according to Bills and Cox (1929), bakers' yeast was utilized as a commercial source of ergosterol. With the discovery that *Saccharomyces carlsbergensis* was characteristically a richer source, this species was adopted commercially.

It has been reasoned by several investigators (Heiduschka and Lindner, 1929; Maguigan and Walker, 1940; Smedley-MacLean, 1928; Smedley-MacLean and Thomas, 1920) that ergosterol, being a lipide, should be increased in cells by cultural practices which favor lipide synthesis. However, Terroine *et al.* (1927) were unable to substantiate this theory, and Massengale and coworkers (1932) concluded from extensive studies that ergosterol synthesis, while not correlated with lipide or protein production or with the carbohydrate reserve in the cells, did show a relationship with the particular carbohydrate used. They classified sugars insofar as their ability to promote synthesis of ergosterol was concerned into the following three groups: (a) xylose, mannitol, α -methyl glucoside, lactose, melibiose, and melizitose, (b) galactose, mannose, fructose, and glucose, and (c) sucrose and maltose. Carbohydrates in group (a) gave cells containing 5% total lipide and 0.5% ergosterol, those in group (b) 1% total lipide and 0.5% ergosterol, and those in group (c) 1.1 to 2.2% lipide and 0.7–0.8% ergosterol. Peculiarly, raffinose, which yeasts hydrolyze to fructose and nonassimilable melibiose, supported a somewhat lower cell crop but the cells contained 1.2% ergosterol. Similarly with regard to the influence of carbohydrate source, Ramaswamy and Sreenivasaya (1946) reported that yeast recovered from maltose fermentations was richer in ergosterol than that from sucrose. Ammonium sulfate also enhanced its formation slightly.

Aerobic cultivation appears to be a prime requisite for ergosterol production by yeasts. This point, about which there is general agreement, is exemplified by the observation of Bills *et al.* (1930) that brewers' yeast normally low in ergosterol could produce up to 1.4% when propagated aerobically. In addition to aerobic conditions Prickett *et al.* (1930) recommended neutral or weakly alkaline conditions. Likewise, Zorkoczy (1942) found that both total lipide and ergosterol were higher in alkaline media whereas the latter was absent in cells propagated at

pH 5.0. It was the opinion of this investigator that conditions which favored the breakdown of yeast total lipides led to the accumulation of ergosterol.

Ascorbic acid when added to a sucrose-phosphate medium heavily seeded with bakers' yeast was observed by Maguigan and Walker to enhance ergosterol formation (1940). Since the medium was actively aerated, the effect of ascorbic acid is not readily explainable. The nitrogen source and supply seemingly play a role in ergosterol synthesis also, since casein hydrolyzate and certain amino acids adversely affected synthesis when added to the above sucrose-phosphate medium.

Gordon has described a process for enriching yeasts in ergosterol which involves restricting the nitrogen supply and aerating in the presence of nontoxic oxidizing agents (1936). As suitable oxidizing agents, such compounds as persulfate, percarbonate, peracetate, perphosphate, hydroquinone, indigo carmine, methylene blue, and peroxides of potassium, sodium, and calcium were claimed. When yeast was cultivated aerobically for nine hours at 92°F. with methylene blue present and with the nitrogen supplement restricted and supplied only during the first half of the fermentation, the resulting cells contained 1.19% ergosterol compared with 0.4% for the control. A further improvement was patented by Gordon (1942) wherein the above process was modified by increasing the temperature to 98°F. for the second half of the fermentation cycle and adding during this stage ethanol or ethanol-lactic acid mixtures. By this procedure cells containing as much as 2.7% ergosterol were produced.

XIII. VITAMIN K

The first indication that microorganisms might contain Vitamin K (antihemorrhagic vitamin), although not recognized as such, was in the work of Anderson and Newman (1933) with tubercule bacilli. They isolated a fat-soluble pigment from *Mycobacterium tuberculosis* and identified it as phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone) which is closely related to synthetic vitamin K (2-methyl-1, 4-naphthoquinone) and since has been shown to have slight antihemorrhagic activity (Almquist and Klose, 1939). In 1935 Almquist and Stokstad (1935) observed that fish meal and rice bran, when stored under conditions favorable to bacterial activity, increased substantially in Vitamin K potency. This was attributed to the synthetic capacities of microorganisms, an hypothesis which was confirmed subsequently by pure culture studies with several species of bacteria (Almquist *et al.*, 1938). The investigations of Dam *et al.* (1941) and Orla-Jensen *et al.* (1941) along with the above make it clear that the elaboration of Vitamin K by

microorganisms is fairly common. The antihemorrhagic principle produced by bacteria has been characterized as vitamin K₂ (McKee *et al.*, 1939; Thayer *et al.*, 1939).

Of various organisms explored, highest potencies have been attained by cells of *Escherichia coli* (Dam *et al.*, 1941). Synthesis by this organism proceeded about equally as well on a synthetic medium of asparagin, citrate, glucose, and salts as on more complex media containing casein hydrolyzate and autolyzed yeast. Under the best conditions *E. coli* cells on a dry basis assayed 1300 to 1600 Dam-Glavind units per gram (equivalent to about 150 to 200 μ g. vitamin K₂/g.). Cells of *Bacterium bifidum*, *Streptococcus fecium*, and *Microbacterium lacticum* were approximately one-fifth as active as *E. coli*. The greater proportion of the vitamin appeared to be retained within the cells. Not all organisms can perform the synthesis, however, since *Pseudomonas aeruginosa* (Almquist *et al.*, 1938), *Lactobacillus acidophilus*, and yeast (Dam, 1942) have been found to be extremely low or devoid of vitamin K.

That Vitamin K is important in the nutrition of certain bacteria is demonstrated by its growth-promoting ability for Johne's bacillus (Woolley and McCarter, 1940). *Mycobacterium phlei* cells have long been employed as a supplement to the medium for culturing Johne's bacillus and thus the activity of vitamin K for this organism indicates that at least one of the stimulatory factors in such cells is due to vitamin K or closely-related compounds. Aside from the above evidence relative to its role in microbial nutrition, Woolley (1945) has observed that a derivative of vitamin K (2-3 dichloro-1, 4-naphthoquinone) is inhibitory to various species. Only with yeast, however, could the inhibition be reversed by the addition of 2 methyl-1, 4-naphthoquinone. While these findings are in a sense analogous to sulfonamide-*p*-aminobenzoic acid relationships, which suggested an essential role for the latter in nutrition, competitive inhibition is not as concise and further studies will be needed to determine the extent of the similarity. Dam *et al.* (1940) were unable to demonstrate that vitamin K influenced either growth or respiration of yeasts.

XIV. CAROTENOIDS

Carotenoids, fat-soluble pigments of the polyene type, are widely distributed among microorganisms. Some have been related to photosynthetic reactions which are common to the purple sulfur bacteria, while others, principally α -, β -, and γ -carotene are of especial interest in view of their vitamin A activity. Whereas vitamin A has not been found, by far the greater number of fungi which produce carotenoids synthesize some β -carotene. Little is known about the other carotenoids present

in microorganisms, largely owing to their complex nature as well as to the lack of suitable methods for their isolation and identification.

Bacteria which have been reported to produce carotenoids include members of the following genera: *Staphylococcus*, *Corynebacterium*, *Mycobacterium*, *Sarcina*, and *Bacterium* (Ingraham, 1936). However, these substances do not appear to be a product of either anaerobes or facultative organisms cultured anaerobically (Ingraham and Baumann, 1934).

Mycobacterium phlei, *Corynebacterium carotenum*, and an unidentified diphtheroid have been found to contain from 0.35 to 0.58 mg. of carotenoid pigments per gram of cells (Ingraham 1935, 1936; Ingraham and Baumann, 1928). At least ten pigments including α - and β -carotene, cryptoxanthin, lutein, and zeoxanthin have been demonstrated in the carotenoid fraction of one of these organisms, *Myco. phlei* (Ingraham, 1935, 1936). When cultured in mineral oil, *Myco. lacticola* formed about 4- μ g. of total carotenoid per ml., about one-fifth of which resembled β -carotene.

Among the yeasts, the *Rhodotorula* group are prolific producers of carotenoids and this characteristic serves to distinguish them from the other nonsporogenous yeasts. From *Rhodotorula rubra*, β -carotene and torulene have been identified (Lederer, 1934) and another species yielded in addition an astacin-like compound (Lederer, 1933). *Rhodotorula sanniei*, according to Fromageot and Tchang (1938a) formed at least eight pigments including β -carotene (10 μ g./g.) and torulene (147 μ g./g.). By biological assay *Rhodotorula glutinis* was found to contain from 5 to 10 I.U. of vitamin A activity (Zirplel, 1941). Some but not all species of *Sporobolomyces*, likewise, synthesize β -carotene as well as other carotenoid pigments (Lederer, 1934).

Of the lower fungi, *Phycomyces blakesleeanus* appears to be the outstanding producer of β -carotene with cells of this organism propagated on synthetic medium having been reported to attain potencies in the neighborhood of 0.25% (Schopfer, 1935). Carotene formation was enhanced by adding asparagin and by exposing cultures to blue or violet light.

In general, the formation and decomposition of carotene pigments by microorganisms parallels lipid storage and utilization. Glycerol, which promotes fat synthesis, is, for example, one of the most favorable carbon sources for carotenoid production. Many of the lower alcohols, notably isopropyl, stimulate, whereas β -ionone, phytol, and even vitamin A are ineffective (Baumann *et al.*, 1933; Ingraham and Baumann, 1934; Ingraham and Steenbock, 1935; Schopfer, 1943). Iron which is essential to the formation of certain pigments likewise does not influence carotenoid synthesis (Zirplel, 1941).

XV. VITAMINS IN FERMENTATION PRODUCTS

Industries which employ as raw materials agricultural commodities frequently encounter serious waste disposal problems. In the fermentation industry these problems are especially challenging because large volumes of waste liquors with low solids but high B.O.D. content are produced. Residual solids from fermentations are comprised largely of the noncarbohydrate portion of the original mash constituents along with cells of the microorganisms involved and their nonvolatile products. Through research on both the biochemical and engineering aspects of waste utilization, the industry has developed processes for converting fermentation residues into valuable feed constituents. Recent developments in this field have been reviewed by Boruff (1947).

Following the alcoholic fermentation of grain mashes and distillation of the alcohol, there remains a residual liquor, "whole stillage," which contains from 5 to 7% solids. Whole stillage is passed over screens and the coarser material thus separated is pressed to remove water further and is dried in rotary driers. This fraction which constitutes approximately one-half of the total stillage solids is known as Distillers' Dried Grains or Light Grains. The material passing through the screens, "thin stillage" is evaporated to a sirup and dried on drum driers. This product known as Dried Solubles is an excellent source of protein and vitamins. For some purposes, thin solubles sirup is mixed with Distillers' Grains and dried to give a product termed Distillers' Dried Grains with Solubles or Dark Grains. Representative vitamin analyses for these and other fermentation residues are contained in Table XII. Of the final potency of distillers' by-products feeds, about two-thirds of the riboflavin, over one-half of the choline, and a small fraction of the niacin are derived from the yeast (Bauernfeind 1944a, 1944b). During the processing, thiamine and pantothenic acid, although known to be produced by yeast, are actually lost probably owing to the high temperatures reached in cooking the mash and the acidic condition of the residues during drying.

The recognition of the high riboflavin content of waste liquors from the butanol fermentation has been discussed earlier. In contrast to ethyl alcohol fermentations wherein only grain residues are totally recovered, riboflavin concentrates are prepared from the butanol fermentation of both molasses and grain. More recently, the fermentation has been adapted to whey to produce riboflavin concentrates as well as neutral solvents (Meade *et al.*, 1945).

The vitamin and protein content of waste liquors from the submerged penicillin fermentation have been examined by Tanner *et al.* (1945).

TABLE XII
Vitamin Content of Fermentation Residues

Fermentation	$\mu\text{g./g. dry substance}$							Refer- ences
	Thiamine	Riboflavin	Pantothenic acid	Niacin	Pyridoxine	Biotin	Choline	
Ethanol								
Corn: solubles	6-9	15-20	29-36	140-160	8-10	2.0-2.4	6000-7000	19
Light grains	1.5	2-3	30-40	1500-2000	
Grains with solubles	3-4	7-10	70-90	4000-5000	20
Wheat: solubles	4.2-10	13-15	44-46.5	210-220	
Light grains	1.3-1.5	2.9-15	6.3-7.8	72-87	
Grains with solubles	3.1-5.9	2.9-4.1	26	150	20
Rye: solubles	3.5-4.0	13.2-14.0	33-35	48-52	
Light grains	1.6-1.9	3.6-3.8	5.5-7.0	18-19	
Grains with solubles	3.0	8.9	34.4	21.0	20
Granular wheat flour: solubles	5.3-6.0	10.9-11.5	23.7-24.2	80-81	
Light grains	2.2-2.6	3.4-4.5	4.4-5.9	42.5-46.8	
Grains with solubles	4.5	8.8	18.7	68.5	
Butanol								
Molasses residue	15	60	300	30	1
Penicillin								
Mycelium (<i>P. chrysogenum</i>)	2.6	39.8-47.5	150-212	107-150	20.9-24.6	0.57-1.5	200, 301
Mycelium-free residue	7.3-29	592-805	232-333	82-144	0.6-0.8	301
Mycelium (<i>P. notatum</i>)	6.9	20	88	180	0.9	200
2,3 Butanediol								
Wheat (<i>A. polymyza</i>)	6.0	153	2
Limed Wheat Solubles (<i>A. aerogenes</i>)	19	19	121	266a

¹ Technical sales bulletin, Commercial Solvents Corporation.

Nearly a tenfold increase in pantothenic acid and a doubling of the pyridoxine concentration were noted in the course of this fermentation. Riboflavin, niacin, and biotin levels remained essentially unchanged. In processing the mash for penicillin recovery the mycelium is first separated and may be dried directly for use in feeds. After extraction of the penicillin, the residual liquor may be evaporated and dried with or without the mycelium. Feeding tests have shown no toxicities (Newell *et al.*, 1947; Tanner *et al.*, 1945).

One of the accomplishments of wartime research was the development of two fermentation processes for the production of 2,3-butanediol, one using *Aerobacillus polymyxa*, the other *Aerobacter aerogenes*. Adams and Leslie (1946) found that in *A. polymyxa* fermentations, riboflavin in the mash was increased from 60 to 106%, averaging about 80%. About 40% of the niacin was destroyed. Biosynthesis coupled with solids losses due to fermentation increases the riboflavin and niacin potencies of the grain solids, respectively, about 5.4 and 1.8 times their original content in wheat. Solubles prepared from the fermentation of wheat with *A. aerogenes* appear to be comparable to Distillers' Solubles (Seagram Research Report, 1944).

In most fermented foods, microorganisms supplement nutritional value through their elaboration of vitamins. Cheese may be taken as an example, although the number of others which might be mentioned for the purpose of illustration is manifold. Burkholder *et al.* (1943) found that thiamine, riboflavin, biotin, and niacin were increased in the surface layers of Camembert, Brie, Liederkrantz, and Limburger cheeses during ripening probably as a result of microbial activity. Aged Swiss and American types were observed by Day and Darby (1936) to contain more riboflavin than cream cheese wherein curing is negligible (6, 8, and 1.4 $\mu\text{g./g.}$ respectively). Appreciable enhancement of pantothenic acid, niacin, and biotin during curing also have been reported by others (Sullivan *et al.*, 1943).

XVI. SUMMARY

On the basis of their sporadic distribution in microorganisms as well as their general lack of essentiality, ascorbic acid and the fat-soluble vitamins and provitamins are readily separable from the regularly accepted members of the B complex. Since analytical methods for the former are neither as sensitive nor as specific as for the B vitamins, it might be argued that their occurrence is more extensive than presently indicated. However, it is difficult to conceive that, aside from the few species which require fat-soluble vitamins, all others including the

exceptionally fastidious types are capable of sufficient synthesis to satisfy possible growth requirements.

A further quantitative difference can be drawn between the two groups of vitamins mentioned above in that, where the fat-soluble vitamins and ascorbic acid are produced they are synthesized in significantly greater amounts. In support of this distinction, the profuse formation of β -carotene by *Phycomyces*, ergosterol by various species, and ascorbic acid by *Aspergillus niger* may be cited.

Contrasted with the fat-soluble factors and ascorbic acid, vitamins of the B group are universally distributed in microorganisms and occur there at a level appreciably above that commonly found in other biological specimens. Likewise, the majority of microbial species are capable of synthesizing these substances in many instances by making use of only the simpler forms of carbon and nitrogen.

While numerous bacteria, yeasts, and molds are capable of a marked degree of vitamin elaboration, yeasts usually attain highest cell potencies because of an added ability to absorb and retain vitamins within their cells. Only *p*-aminobenzoic acid is excreted in substantial amounts by the yeasts which have been studied, whereas bacteria and molds release the greater proportion of that fabricated.

In view of the fact that B vitamins have now been definitely associated as coenzymes in both anabolic and catabolic processes, it becomes of interest to attempt a correlation of these functions with vitamin formation and content. Though data are necessarily limited, there are suggestions of such a relationship. For example, thiamine, niacin, and pantothenic acid which are involved in fermentative mechanisms are generally either synthesized or absorbed more actively by anaerobically-propagated cells. On the other hand folic acid, biotin, *p*-aminobenzoic acid, and pyridoxine which participate in anabolic reactions generally are produced in greater amounts by aerobically-propagated cells. This is in accord with the enhanced assimilative activity under aerobic conditions. Exceptions such as the unusual production of pantothenic acid by *Penicillium chrysogenum* and thiamine by *Azotobacter chroococcum* under strongly aerobic conditions, however, serve to emphasize the difficulties inherent in attempting to differentiate between vitamin requirements for anabolic and catabolic reactions.

With the exception of synthesis from precursors, the production of extraordinary amounts of vitamins in most instances is associated with abnormal environmental conditions. For example, ergosterol formation proceeds at a maximum rate only when nitrogen is restricted and strongly aerobic conditions prevail. Similarly, *Candida* species elaborate ribo-

flavin only when iron levels are adjusted below that which is optimum for growth. The resulting formation of vitamins under such conditions may occur because the organism resorts to alternative metabolic pathways. The accumulation of vitamins also is akin to the blocking of synthesis in certain mutants and suggests approaches which might be applied on a broader basis to increase the production of nitrilites. However, the utilization of antivitamins to promote synthesis has not met with marked success.

Investigations on the quantitative synthesis of vitamins by microorganisms has been limited both with reference to the number of species and to the influence of conditions. It is apparent, nevertheless, that this approach is capable of providing much information of both theoretical and practical significance.

REFERENCES

1. Abdel—Salaam, A., and Leong, P. C. 1938. *Biochem. J.* **32**, 958-963.
2. Adams, G. A., and Leslie, J. D. 1946. *Can. J. Research* **24F**, 12-28.
3. Agarwal, P. N., Singh, K., King, P. S., and Peterson, W. H. 1947. *Arch. Biochem.* **14**, 105.
4. Allinson, M. J. C. 1943. *J. Biol. Chem.* **147**, 785-791.
5. Almquist, H. J., and Klose, A. A. 1939. *J. Am. Chem. Soc.* **61**, 1611.
6. Almquist, H. J., Pentler, C. F., and Mecchi, E. 1938. *Proc. Soc. Exptl. Biol. Med.* **38**, 336-338.
7. Almquist, H. J., and Stokstad, E. L. R. 1935. *J. Biol. Chem.* **111**, 105-113.
8. Anderson, R. J. 1932. *Physiol. Rev.* **12**, 166-189.
9. Anderson, R. J., and Newman, M. S. 1933. *J. Biol. Chem.* **103**, 197-201.
10. Anderson, R. J., Schoenheimer, R., Crowder, J. A., and Stodola, F. A. 1931. *Z. physiol. Chem.* **237**, 40-45.
11. Angier, R. B., Boothe, J. H., Hutchings, B. L., Mowat, J. H., Semb, J., Stokstad, E. L. R., SubbaRow, Y., Waller, C. W., Cosulich, D. B., Fahrenback, M. J., Hulquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1945. *Science* **102**, 227-228.
12. Ansbacher, S. A. 1944. *Vitamins and Hormones* **2**, 215-247.
13. Arzberger, C. F. 1943. U. S. Patent 2,326,425.
14. Ashida, K. 1942. *J. Agr. Chem. Soc. Japan* **18**, 723-726.
15. Atkin, L., Schultz, A. S., Williams, S. L., and Frey, C. N. 1943. *Ind. Eng. Chem., Anal. Ed.* **15**, 141-144.
16. Axelrod, A. E., DeWoody, J., and Hofmann, K. 1946. *J. Biol. Chem.* **163**, 771.
17. Axelrod, A. E., Flinn, B. C., and Hofmann, K. 1947. *J. Biol. Chem.* **169**, 195-202.
18. Barker, H. A., and Peterson, W. H. 1944. *J. Bact.* **47**, 307-308.
19. Bauernfeind, J. C., Garey, J. C., Baumgarten, W., Stone, L., and Boruff, C. S., 1944a. *Ind. Eng. Chem.* **36**, 76-78.
20. Bauernfeind, J. C., Smith, M. B., Garey, J. C., Baumgarten, W., Gustoff, F. H and Stone, L. 1944b. *Cereal Chem.* **21**, 421-429.
21. Baumann, C. A., Steenbock, H., Ingraham, M. A., and Fred, E. B. 1933. *J. Biol. Chem.* **103**, 339.

- 21a. Beadle, G. W., Mitchell, H. K., and Nye, J. F. 1947. *Proc. Natl. Acad. Sci. U.S.* **33**, 155-158.
22. Bellamy, W. D., Umbreit, W. W., and Gunsalus, I. C. 1945. *J. Biol. Chem.* **160**, 461-472.
23. Bernhauer, K., Gorlich, O., and Kocher, E. 1936. *Biochem. Z.* **286**, 60-65.
24. Bills, C. E. 1935. *Physiol. Revs.* **15**, 1-97.
25. Bills, C. E., and Cox, W. M., Jr. 1929. *J. Biol. Chem.* **84**, 455-462.
26. Bills, C. E., Massengale, O. N., and Prickett, P. S. 1930. *J. Biol. Chem.* **87**, 259-264.
27. Bina, A. F., Thomas, J. M., and Brown, E. B. 1943. *J. Biol. Chem.* **148**, 111-116.
28. Bird, O. D. 1947. *Nature* **159**, 33.
29. Birkinshaw, J. H., Callow, R. K., and Fischmann, C. F. 1931. *Biochem. J.* **25**, 1977.
30. Blanchard, K. C. 1941. *J. Biol. Chem.* **140**, 919.
31. Boas-Fixen, M. A., and Roscoe, M. H. 1940. *Nutrition Abstracts & Revs.* **9**, 795-861.
32. Bohonos, N., Hutchings, B. L., and Peterson, W. H. 1942. *J. Bact.* **44**, 479-485.
33. Boissevain, C. H., Drea, W. F., and Schultz, H. W. 1938. *Proc. Soc. Exptl. Biol. Med.* **39**, 481-483.
- 33a. Bonner, D. 1948. *Proc. Natl. Acad. Sci. U.S.* **34**, 5-9.
34. Bonner, D., and Beadle, G. W. 1946. *Arch. Biochem.* **11**, 319-328.
35. Bonner, J., and Buchman, E. R. 1939. *Proc. Natl. Acad. Sci. U.S.* **25**, 164-171.
36. Boruff, C. S. 1947. *Ind. Eng. Chem.* **39**, 602-7.
37. Bovornick, M. R. 1943. *J. Biol. Chem.* **151**, 467-75.
38. Bourne, G., and Allen, R. J. L. 1935. *Australian J. Exptl. Biol. Med. Sci.* **13**, 165-174.
39. Bunker, H. F. 1947. *Chemistry & Industry* 203-205.
40. Burkholder, P. R. 1943a. *Proc. Natl. Acad. Sci. U. S.* **29**, 166-172.
41. Burkholder, P. R. 1943b. *Arch. Biochem.* **3**, 121-129.
42. Burkholder, P. R. 1944. U. S. Patent 2,363,277.
43. Burkholder, P. R., Collier, J., and Moyer, D. 1943. *Food Research* **8**, 314-322.
44. Burkholder, P. R., and McVeigh, I. 1942. *Proc. Natl. Acad. Sci. U. S.* **28**, 285-289.
45. Burkholder, P. R., McVeigh, I., and Wilson, K. 1945. *Arch. Biochem.* **1**, 287-303.
46. Büsing, K. H., and Peters, F. 1940. *Biochem. z.* **304**, 134-136.
47. Carpenter, C. C., and Friedlander, E. W. 1942. *Science* **95**, 625.
48. Cavallito, C. J. 1944. *Science* **100**, 333.
49. Cheldelin, V. H., Hoag, E. H., and Sarett, H. P. 1945. *J. Bact.* **49**, 41-45.
50. Crowe, M. O. L. 1939. *Proc. Soc. Exptl. Biol. Med.* **42**, 212-215.
51. Cuffaro, M. 1940. *Boll. Soc. ital. biol. sper.* **15**, 966-968.
52. Daft, F. S., and Sebrell, W. H. 1945. *Vitamins and Hormones* **3**, 49-69.
53. Dam, H. 1942. *Advances in Enzymol.* **2**, 285-324.
54. Dam, H., Glavind, J., and Nielsen, N. 1940. *Z. physiol. Chem.* **265**, 80-87.
55. Dam, H., Glavind, J., Orla-Jensen, S., and Orla-Jensen, A. D. 1941. *Naturwissenschaften* **29**, 287-288.
56. Damon, S. R. 1923. *J. Biol. Chem.* **56**, 895-902.
57. Day, P. L., and Darby, W. J. 1936. *Food Research* **1**, 349-355.
58. Dittmer, K., Melville, D. B., and du Vigneaud, V. 1944. *Science* **99**, 203-205.

59. Dorfman, A., Berkman, S., and Koser, S. A. 1942. *J. Biol. Chem.* **144**, 393-400.
60. Duschinsky, R., Dolan, L. A., Flower, D., and Rubin, S. H. 1945. *Arch. Biochem.* **8**, 480-481.
61. Eakin, R. E., and Eakin, E. A. 1942. *Science* **96**, 187.
62. Eakin, R. E., Snell, E. E., and Williams, R. J. 1941. *J. Biol. Chem.* **140**, 535-543.
63. Eakin, R. E., and Williams, R. J. 1940. *J. Biol. Chem.* **146**, 801-802.
64. Eastcott, E. V. 1928. *J. Phys. Chem.* **32**, 1094-1111.
65. Eijkman, C., Van Hoogenhuyze, C. J., and Derks, T. J. G. 1922. *J. Biol. Chem.* **60**, 311-314.
66. Ekstrand, T., and Sjorgren, B. 1945. *Nature* **156**, 476-477.
67. Eppright, M. A., and Williams, R. J. 1946. *J. Gen. Physiol.* **30**, 61-72.
68. von Euler, H., Hoberg, B., Karrer, P., Salomon, H., and Ruckstuhl, H. 1944. *Helv. Chim. Acta* **27**, 382-390.
69. Fawns, H., and Jung, A. 1933. *Biochem. Z.* **27**, 918-933.
70. Fink, H., and Just, F. 1939. *Biochem. Z.* **303**, 404-414.
71. Fink, H., and Just, F. 1941a. *Biochem. Z.* **308**, 15-28.
72. Fink, H., and Just, F. 1941b. *Biochem. Z.* **309**, 219-237.
73. Fink, H., and Just, F. 1942a. *Biochem. Z.* **311**, 61-72.
74. Fink, H., and Just, F. 1942b. *Biochem. Z.* **311**, 287-306.
75. Fink, H., and Just, F. 1942c. *Biochem. Z.* **313**, 39-47.
76. Fink, H., Just, F., and Hoch, A. 1942. *Ber.* **75B**, 2101-2110.
77. Fischer, A. M. 1938. *Brewers Digest* **13**, 189-90.
78. Foster, J. W. 1947. British Patent 593,027.
- 78a. Fromageot, C., and Tchang, J. L. 1938a. *Arch. Mikrobiol.* **9**, 424-433.
- 78b. Fromageot, C., and Tchang, J. L. 1938b. *Arch. Mikrobiol.* **9**, 433-448.
- 78c. Galli, A. 1946. *Ber. schweiz. botan. Ges.* **56**, 113-74; *Chem. Abstracts* **42**, 2639c (1948).
79. Garrison, L. S., and Eakin, R. E., Unpublished data, quoted by Shive and Rogers, 1947.
80. Geiger-Huber, M., and Galli, H. 1945. *Helv. Chim. Acta* **28**, (1), 248-250.
81. Genung, E. F., and Lee, M. E. 1944. *J. Bact.* **47**, 434-435.
82. Gerard, E. 1892. *Compt. rend.* **114**, 1544.
83. Gerard, E. 1895a. *Compt. rend.* **121**, 724.
84. Gerard, E. 1895. *J. pharm. chim.* **1**, 601.
85. Gerard, E. 1898. *Compt. rend.* **126**, 909.
86. Gorcica, H. J., and Levine, H. 1942. U. S. Patent 2,295,036.
87. Gordon, W. G. 1936. U. S. Patent 2,059,980.
88. Gordon, W. G. 1942. U. S. Patent 2,776,710.
89. Gottlieb, D., and Gilligan, G. M. 1946. *Arch. Biochem.* **10**, 163-164.
90. Guha, B. C. 1932. *Indian J. Med. Res.* **19**, 977-983.
91. Guilliermond, A. 1928. *Rev. gén. Botan.* **40**, 606-624; 690-704.
92. Guilliermond, A. 1936. *Rev. Mycol.* NS **1**, 115-156.
93. Guilliermond, A., Fontaine, M., and Raffy, A. 1935. *Compt. rend.* **201**, 1077-1080.
94. Haas, H. F., and Bushnell, L. D. 1944. *J. Bact.* **48**, 219-231.
95. Handler, P., and Klein, J. R. 1942. *J. Biol. Chem.* **143**, 49-51.
96. Harden, A., and Young, W. J. 1906. *Proc. Roy. Soc. London* **77B**, 405-420; 1906, **78B**, 369-375.

97. Harington, C. R., and Moggridge, R. C. G. 1940. *Biochem. J.* **34**, 685-689.
98. Harris, S. A., Heyl, D., and Folkers, K. 1944. *J. Biol. Chem.* **154**, 315-316.
99. Harrison, H. E. 1944. U. S. Patent 2,359,521.
100. Hartelius, V. 1946. *Compt. rend. trav. lab. Carlsberg, Ser. physiol.* **24**, 178-184.
- 100a. Hecht, E. 1935. *Z. physiol. chem.* **231**, 29-38.
101. Heiduschka, A., and Lindner, H. 1929. *Z. physiol. chem.* **181**, 15-23.
102. Herrick, J. A., and Alexopoulos, C. J. 1943. *Bull. Torrey Botan. Club.* **70**, 369-371.
103. Hickey, R. J. 1945. *Arch. Biochem.* **8**, 439-447.
104. Hickey, R. J. 1947. U. S. Patent 2,425,280.
105. Hills, G. M. 1943. *Biochem. J.* **37**, 418-435.
106. Hochberg, M., Melnick, D., and Oser, B. L. 1945. *J. Nutrition* **30**, 201-208.
107. Hofmann, K. 1943. *Advances in Enzymology* **3**, 289-313.
108. Hofmann, K. 1945. *J. Am. Chem. Soc.* **67**, 694.
109. Hofmann, K., and Axelrod, A. E. 1946. *Arch. Biochem.* **11**, 376-377.
110. Hofmann, K., and Winnick, T. 1945. *J. Biol. Chem.* **160**, 449-453.
111. Hofmann, K., Winnick, T., and Axelrod, A. E. 1947. *J. Biol. Chem.* **169**, 191-193.
112. Hogan, A. G., and Kamm, O. 1943. *Science* **97**, 353.
113. Hutchings, B. L., Stokstad, E. L. R., Bohonos, N., Sloane, N. H., and SubbaRow, Y. 1946a. *Ann. New York Acad. Sci.* **48**, 265-267.
114. Hutchings, B. L., Stokstad, E. L. R., Mowat, J. H., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y. 1946b. *Ann. New York Acad. Sci.* **48**, 273-277.
- 114a. Ingraham, M. A. 1935. *J. Bact.* **29**, 74.
- 114b. Ingraham, M. A. 1936. *J. Bact.* **31**, 18-19.
115. Ingraham, M. A., and Baumann, C. A. 1928. *J. Bact.* **28**, 31-40.
116. Ingraham, M. A., and Baumann, C. A. 1934. *J. Bact.* **27**, 25-26.
117. Ingraham, M. A., and Steenbock, H. 1935. *Biochem. J.* **29**, 2553-2562.
118. Jukes, T. H., and Stokstad, E. L. R. 1947. *J. Biol. Chem.* **168**, 563-567.
- 118a. Karrer, P., and Rutschman, J. 1943. *Helv. Chim. Acta* **26**, 2109-2114.
- 118b. Karrer, P., and Rutschman, J. 1945. *Helv. Chim. Acta* **28**, 795-797.
119. Kavanagh, F. 1942. *Bull. Torrey Botan. Club* **69**, 669-691.
120. Keresztesy, J. C., Rikes, E. L., and Stokes, J. L. 1943. *Science* **97**, 465.
121. Kidder, G. W., and Dewey, V. C. 1942. *Growth* **6**, 405-418.
122. Knight, B. C. J. G. 1937. *Biochem. J.* **31**, 966-973.
123. Knight, B. C. J. G. 1945. *Vitamins and Hormones* **3**, 108-217.
124. Knoblock, H., and Sellman, R. 1941. *Zentr. Bakt. Parasitenk.* **II**, **103**, 277-280.
125. Kögl, F. 1935. *Ber.* **68**, 16-28.
126. Kögl, F., and Fries, N. 1937. *Z. physiol. chem.* **249**, 93-110.
127. Kögl, F., and Tönnis, B. 1936. *Z. physiol. chem.* **242**, 43-73.
128. Koser, S. A., and Baird, G. R. 1944. *J. Infectious Diseases* **75**, 250-261.
129. Krampitz, L. O., and Woolley, D. W. 1944. *J. Biol. Chem.* **152**, 9-17.
130. Kroecker, E. H., Strong, F. M., and Peterson, W. H. 1935. *J. Am. Chem. Soc.* **57**, 354-356.
- 130a. Krueger, K. K., and Peterson, W. H. 1948. *J. Bact.* **55**, 693-703.
131. Kuhn, R., and Wieland, T. 1942. *Ber.* **75B**, 121-123.
132. Kurth, E. F., and Cheldelin, V. H. 1946. *Ind. Eng. Chem.* **38**, 617-619.
133. Lampen, J. O., Baldwin, I. L., and Peterson, W. H. 1945. *Arch. Biochem.* **7**, 277-286.

134. Lampen, J. O., and Jones, M. J. 1947. *J. Biol. Chem.* **170**, 133-146.
135. Lampen, J. O., Roepke, R. R., and Jones, M. J. 1946. *J. Biol. Chem.* **164**, 789-790.
136. Landy, M., and Dicken, D. 1941. *Proc. Soc. Exptl. Biol. Med.* **46**, 449-452.
137. Landy, M., and Dicken, D. 1942. *Nature* **149**, 244.
138. Landy, M., and Dicken, D. 1942. *J. Biol. Chem.* **146**, 109-114.
139. Landy, M., Dicken, D., Bicking, M. M., and Mitchell, W. R. 1942. *Proc. Soc. Exptl. Biol. Med.* **49**, 441-444.
140. Landy, M., and Gerstung, R. B. 1945. *J. Immunol.* **51**, 269-277.
141. Landy, M., Larkum, N. W., Oswald, E. J., and Streightoff, F. 1943a. *Science* **97**, 265-267.
- 141a. Landy, M., Larkum, N. W., and Oswald, E. J. 1943b. *Proc. Soc. Exptl. Biol. Med.* **52**, 338.
142. Landy, M., and Streightoff, F. 1943. *Proc. Soc. Exptl. Biol. Med.* **52**, 127-128.
143. Lardy, H. A., Potter, R. L., and Elvehjem, C. A. 1947. *J. Biol. Chem.* **169**, 251-252.
144. Lavollay, J., and Laborey, F. 1937. *Compt. rend.* **205**, 174-180.
145. Lavollay, J., and Laborey, F. 1938. *Compt. rend.* **206**, 1055-1056.
146. Lavollay, J., and Laborey, F. 1939. *Compt. rend.* **208**, 1056-1058.
147. Lavollay, J., and Laborey, F. 1941. *Ann. fermentations* **6**, 129-142.
148. Lederer, E. 1933. *Compt. rend.* **197**, 1694-1695.
- 148a. Lederer, E. 1934. *Compt. rend. soc. biol.* **117**, 1083-1085.
149. Lee, S. B., and Burris, R. H. 1943. *Ind. Eng. Chem.* **35**, 354-357.
150. Legg, D. A., and Beesch, S. C. 1945. U. S. Patent 2,370,177.
151. Lemberg, R., Tandy, D., and Goldsworthy, N. E. 1946. *Nature* **157**, 103.
152. Leonian, L. H., and Lilly, V. G. 1943. *J. Bact.* **45**, 329-339.
153. Leonian, L. H., and Lilly, V. G. 1945. *J. Bact.* **49**, 291-297.
154. Leviton, A. 1946. *J. Am. Chem. Soc.* **68**, 835-840.
155. Lewis, J. C. 1942. *J. Biol. Chem.* **146**, 441-450.
156. Lewis, J. C. 1944. *Arch. Biochem.* **4**, 217-228.
157. Lewis, J. C., Stubbs, J. J., and Noble, W. M. 1944. *Arch. Biochem.* **4**, 389-401.
158. Lewis, R. W. 1939. *Papers Mich. Acad. Sci.* **24**, Pt. 1, 31-35.
159. Lichstein, H. C., Gunsalus, I. C., and Umbreit, W. W. 1945. *J. Biol. Chem.* **161**, 311-320.
160. Lichstein, H. C., and Umbreit, W. W. 1947a. *J. Biol. Chem.* **170**, 329-336.
161. Lichstein, H. C., and Umbreit, W. W. 1947b. *J. Biol. Chem.* **170**, 423-424.
162. Lillie, R. J., and Briggs, G. M., Jr. 1947. *Poultry Sci.* **26**, 289-294.
163. Lilly, V. G., and Leonian, L. H. 1944. *Science* **99**, 205-206.
164. Lipmann, F., and Kaplan, N. O. 1946. *Federation Proc.* **5**, 145.
165. Lipmann, F., Kaplan, N. O., and Novelli, G. D. 1947a. *Federation Proc.* **6**, 272.
166. Lipmann, F., Kaplan, N. O., Novelli, G. D., Tuttle, L. C., and Guirard, B. M. 1947b. *J. Biol. Chem.* **167**, 868-870.
167. Lipmann, F., and Tuttle, L. C. 1945. *J. Biol. Chem.* **161**, 415-416.
168. Livshits, M. I. 1941. *Proc. Sci. Inst. Vitamin Research U.S.S.R.* **3**, 184-188.
169. Lunde, G., and Lie, J. 1938. *Z. physiol. Chem.* **254**, 227.
170. Lutz, A. 1947. *Experimentia* **3**, 244-245.
171. Lyman, C. M., Moseley, O., Wood, S., Butler, B., and Hale, F. 1946. *J. Biol. Chem.* **162**, 173-174.
172. MacDonough, J. V., and Haffenreffer, T. C., Jr. 1944. *Wallerstein Labs. Commun.* **7**, 39-46.

173. Maguigan, W. A., and Walker, E. 1940. *Biochem. J.* **34**, 804-813.
174. Maizel, B. 1946. U. S. Patent, 2,411,445.
175. Massengale, O. N., Bills, C. E., and Prickett, P. S. 1932. *J. Biol. Chem.* **94**, 213-219.
176. Massock, H. E. 1943. M.S. Thesis, University of Wisconsin.
177. Massock, H. E., and Baldwin, I. L. 1943. *J. Bact.* **45**, 34.
178. Mayer, R. L. 1946. French Patent 913,165.
179. Mayer, R. L., and Rodbart, R. 1946. *Arch. Biochem.* **11**, 49-63.
180. McIntire, F. C., Riker, A. J., and Peterson, W. H. 1941. *J. Bact.* **42**, 1-13.
181. McKee, R. W., Binkley, S. B., MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A. 1939. *J. Am. Chem. Soc.* **61**, 1295.
182. Mead, M. W., Jr., and Lee, J. 1943. U. S. Patent 2,328,025.
183. Meade, R. E., Pollard, H. L., and Rodgers, N. E. 1945. U. S. Patent 2,369,680.
- 183a. Meade, R. E., Rodgers, N. E., and Pollard, H. L., 1947, U. S. Patent 2,433,232.
184. Melnick, D., Hochberg, M., Himes, H. W., and Oser, B. L. 1945. *J. Biol. Chem.* **160**, 1-14.
185. Melville, D. B. 1944. *Vitamins and Hormones* **2**, 29-69.
186. Melville, D. B., Dittmer, K., Brown, G. B., and du Vigneaud, V. 1943. *Science* **98**, 497-499.
187. Melville, D. B., Hofmann, K., Hague, E., and du Vigneaud, V. 1942. *J. Biol. Chem.* **142**, 615-618.
188. Miller, A. K. 1944. *Proc. Soc. Exptl. Biol. Med.* **57**, 151-153.
189. Mills, R. C., Briggs, G. M., Jr., Luckey, T. D., and Elvehjem, C. A. 1944. *Proc. Soc. Exptl. Biol. Med.* **56**, 240-242.
190. Miner, C. S. 1940. U. S. Patent 2,202,161.
191. Mirimanoff, A., and Raffy, A. 1938a. *Bull. soc. chim. biol.* **20**, 1166.
192. Mirimanoff, A., and Raffy, A. 1938b. *Compt. rend.* **206**, 1507-1509.
193. Mirimanoff, A., and Raffy, A. 1938c. *Helv. Chim. Acta.* **21**, 1004-1006.
- 193a. Mitchell, H. K., and Nyc, J. F. 1948. *Proc. Natl. Acad. Sci. U. S.* **34**, 1-5.
- 193b. Moore, H. N., and deBeeze, G. 1947. *J. Bact.* **54**, 40-41.
194. Morel, M. 1941. *Ann. inst. Pasteur* **67**, 285-298.
195. Mueller, J. H. 1933. *Proc. Soc. Exptl. Biol. Med.* **36**, 706-.
196. Mueller, J. H., and Klotz, A. W. 1938. *J. Am. Chem. Soc.* **60**, 3086-3087.
197. Myrbäck, K., and Vallin, I. 1944. *Svensk. Kem. Tid.* **56**, 400-408.
198. Najjar, V. A., and Barrett, R. 1945. *Vitamins and Hormones* **3**, 23-44.
199. National Research Council, Mimeo. report, Feb. 5, 1943.
200. Newell, G. W., Peterson, W. H., and Elvehjem, C. A. 1947. *Poultry Sci.* **26**, 284-288.
201. Nilsson, R., Enebo, L., and Lundin, H. 1941. *Svensk. Papperstidn.* **44**, 371.
- 201a. Nilsson, R., Enebo, L., and Brunius, E. 1942. *Svensk. Kem. Tid.* **54**, 134-135.
202. Novak, A. F., Stark, W. H., and Kolochoy, P. 1943. *J. Bact.* **45**, 34.
203. Novelli, G. D., and Lipmann, F. 1947a. *Arch. Biochem.* **14**, 23-27.
204. Novelli, G. D., and Lipmann, F., 1947b. *J. Bact.* **54**, 19.
205. Odintsova, E. N. 1941. *Microbiology U.S.S.R.* **10**, 670-687.
206. Odintsova, E. N. 1943. *Compt. rend. acad. sci. U.R.S.S.* **41**, 250-251.
207. O'Kane, D. 1942. *J. Bact.* **43**, 7.
208. Orla-Jensen, S., Orla-Jensen, A. D., Dam, H., and Glavind, J. 1941. *Zentr. Bakt. Parasitenk.* **II**, 104, 202-204.
209. Oxford, A. E., and Raistrick, H. 1933. *Biochem. J.* **27**, 1176-1180.
210. Parsons, H. T., Foeste, A., and Gilberg, H. 1945. *J. Nutrition* **29**, 383-389.

211. Pavcek, P. L., Peterson, W. H., and Elvehjem, C. A. 1937. *Ind. Eng. Chem.* **29**, 536-541.
212. Pavcek, P. L., Peterson, W. H., and Elvehjem, C. A. 1938. *Ind. Eng. Chem.* **30**, 802-805.
213. Pavcek, P. L., Peterson, W. H., Elvehjem, C. A., Saudek, E. E., Colingsworth, D. H., and Baldwin, I. L. 1936. *Wisc. Agr. Expt. Sta. Bull.* No. 435, 80-83.
214. Peck, R. L., and Hansen, C. R. 1940. *J. Biol. Chem.* **134**, 403-412.
- 214a. Peltier, G. L., and Borchers, R. 1947. *J. Bact.* **54**, 519-520.
215. Peterson, W. H., and Peterson, M. S. 1945. *Bact. Revs.* **9**, 49-109.
- 215a. Pett, L. B. 1935. *Biochem. J.* **29**, 937-944.
216. Pfiffner, J. J., Binkley, S. B., Bloom, E. S., and O'Dell, B. L. 1947. *J. Am. Chem. Soc.* **69**, 1476-1487.
217. Pfiffner, J. J., Calkins, D. G., Bloom, E. S., and O'Dell, B. L. 1946. *J. Am. Chem. Soc.* **68**, 1392.
218. Pfiffner, J. J., and Hogan, A. G. 1946. *Vitamins and Hormones* **4**, 1-31.
- 218a. Pfizer, Chas., and Co., Inc. 1948. British Patent 593,953.
219. Piersma, H. D. 1945. U. S. Patent 2,400,710.
220. Pilgrim, F. J., Axelrod, R. E., Winnick, T., and Hofmann, K. 1945. *Science* **102**, 35-36.
- 220a. Pollard, H. L., Rodgers, N. E., and Meade, R. E. 1947. U. S. Patent 2,432,063.
221. Preuss, L. M., Peterson, W. H., Steenbock, H., and Fred, E. B. 1931. *J. Biol. Chem.* **90**, 369-384.
222. Prickett, P. S., Massengale, O. N., Cox, W. M., Jr., and Bills, C. E. 1930. *Proc. Soc. Exptl. Biol. Med.* **27**, 701-702.
223. Proskuryakov, N. I., and Pavlinova, O. A. 1945. *Compt. rend. acad. sci. U.R.S.S.* **47**, 283-285.
224. Rabinowitz, J. C., and Snell, E. E. 1947. *Ind. Eng. Chem., Anal. Ed.* **19**, 277-280.
225. Raffy, A. 1937. *Compt. rend. soc. biol.* **126**, 875-877.
226. Raffy, A. 1939. *Compt. rend.* **209**, 900-902.
227. Raffy, A., and Fontaine, M. 1937. *Compt. rend.* **205**, 1005-1006.
228. Ramaswamy, S., and Sreenivasaya, M. 1946. *J. Sci. Ind. Res. India* **5B**, 51-52.
229. Ratner, S., Blanchard, M., and Green, D. E. 1946. *J. Biol. Chem.* **164**, 691-701.
230. Ravel, J. M., and Shive, W. 1946. *J. Biol. Chem.* **166**, 407-415.
231. Reichelt, E. 1941. *Monatsschr. Kinderheilk.* B.C.A., May 1941, 374.
232. Renaud, J., and Lachaux, M. 1944. *Compt. rend.* **219**, 498-500.
233. Renaud, J., and Lachaux, M. 1945. *Compt. rend.* **221**, 187-188.
234. Robbins, W. J., and Schmidt, M. B. 1939. *Bull. Torrey Botan. Club* **66**, 139-150.
235. Robbins, W. J., and Ma, R. 1942. *Science* **96**, 406-407.
236. Rodgers, N. E. 1942. Ph.D. Thesis, University of Wisconsin.
- 236a. Rodgers, N. E., Pollard, H. L., and Meade, R. E. 1947. U. S. Patent 2,432,064.
237. Rogers, L. L., and Shive, W. 1947. *J. Biol. Chem.* **169**, 57.
238. Rohner, F., and Roulet, F. 1939. *Biochem. Z.* **300**, 148-152.
239. Rubbo, S. D., and Gillespie, J. M. 1940. *Nature* **146**, 838-839.
240. Rubin, S. H., Flower, D., Rosen, F., and Drecker, L. 1945. *Arch. Biochem.* **8**, 79-80.
241. Rudert, F. J. 1945. U. S. Patent 2,374,503.

242. Ryan, F. J., Ballentino, F., Stolovy, E., Corson, M. E., and Schneider, L. K. 1945. *J. Am. Chem. Soc.* **67**, 1857-1858.
243. Sakurai, K. 1940. *J. Sci. Hiroshima Univ. Ser. B. Div. 2* **4**, 1-6.
244. Sarett, H. P. 1947. Abstracts 111th meeting A.C.S. April 1947, pp. 14-15B.
245. Sarma, P. S., Snell, E. E., and Elvehjem, C. A. 1946. *J. Nutrition* **33**, 121-128.
246. Saunders, A., and McClung, L. S. 1943. *J. Bact.* **46**, 575.
247. Savard, K., and Grant, G. A. 1946. *Science* **104**, 459-460.
248. Scheunert, A. 1941. *Biedermann's Zentr. B. Tierernähr.* **13**, 329-340.
249. Scheunert, A., and Schiebllich, M. 1927. *Biochem. Z.* **184**, 58-66.
250. Scheunert, A., and Schiebllich, M. 1935. *Z. Vitaminforsch.* **4**, 294-299.
251. Scheunert, A., and Schiebllich, M. 1936. *Biochem. Z.* **286**, 66-71.
252. Scheunert, A., Wagner, K. H., Fink, H., and Krebs, J. 1939. *Biochem. Z.* **302**, 1-11.
253. Schivek, A. I. 1944. U. S. Patent 2,359,443.
254. Schlenk, F., and Snell, E. E. 1945. *J. Biol. Chem.* **157**, 425-426.
255. Schonberg, K., and Sperber, E. 1945. *Svensk. Kem. Tid.* **57**, 117-123.
256. Schopfer, W. H. 1943. *Plants and Vitamins*. Chronica Botanica Co., Waltham, Mass.
- 256a. Schopfer, W. H. 1935. *Compt. rend. soc. biol.* **118**, 3-5.
257. Schopfer, W. H. 1944. *Helv. Chim. Acta* **27**, 1017-1032.
258. Schopfer, W. H., and Guilloud, M. 1945a. *Experientia* **1**, 22-23.
259. Schopfer, W. H., and Guilloud, M. 1945b. *Experientia* **1**, 332-334.
260. Schultz, A. S., Atkin, L., and Frey, C. N. 1938. *J. Am. Chem. Soc.* **60**, 490.
261. Schultz, A. S., Atkin, L., and Frey, C. N. 1941. U. S. Patent 2,262,735.
262. Schultz, A. S., Atkin, L., and Frey, C. N. 1942. U. S. Patent 2,285,465.
263. Schultz, A. S., Atkin, L., and Frey, C. N. 1944. U. S. Patent 2,354,281.
264. Schultz, A. S., Atkin, L., and Frey, C. N. 1945. U. S. Patent 2,377,044.
265. Schultz, E. M. 1947. *J. Am. Chem. Soc.* **69**, 1056-1057.
266. Schwarz, R., Laufer, S., Laufer, L., and Brenner, M. W. 1942. *Ind. Eng. Chem.* **34**, 480-483.
- 266a. Seagram, Jos. E. and Sons, Sept. 1944. Research Report No. SC 759.
267. Sevag, M. G. 1946. *Advances in Enzymol.* **6**, 33-127.
268. Sevag, M. G., and Green, M. N. 1944. *J. Bact.* **47**, 450, 451.
269. Shive, W., and Rogers, L. L. 1947. *J. Biol. Chem.* **169**, 453-454.
270. Siegel, L., Melnick, D., and Oser, B. L. 1943. *J. Biol. Chem.* **149**, 361-367.
271. Silverman, M., and Werkman, C. H. 1939. *J. Bact.* **38**, 25-32.
272. Singh, K., Agarwal, G. N., and Peterson, W. H. 1947. Abstracts 111th A.C.S. meeting, April 1947, pp. 9-10A.
273. Smedley-MacLean, I. 1928. British Patent 295,757.
274. Smedley-MacLean, I., and Hoffert, D. 1923. *Biochem. J.* **17**, 720-741.
275. Smedley-MacLean, I., and Thomas, E. M. 1920. *Biochem. J.* **14**, 483-493.
276. Snell, E. E. 1944a. *Proc. Soc. Exptl. Biol. Med.* **55**, 36-39.
277. Snell, E. E. 1944b. *J. Biol. Chem.* **154**, 313-314.
278. Snell, E. E. 1945. *J. Biol. Chem.* **157**, 491-505.
279. Snell, E. E., and Guirard, B. M. 1943. *Proc. Natl. Acad. Sci. U. S.* **29**, 66-73.
280. Snell, E. E., Guirard, B. M., and Williams, R. J. 1942. *J. Biol. Chem.* **143**, 519-530.
281. Snell, E. E., Strong, F. M., and Peterson, W. H. 1938. *J. Am. Chem. Soc.* **69**, 2825.
282. Sperber, E. 1942. *Biochem. Z.* **313**, 62-74.
283. Sperber, E., and Renvall, S. 1941. *Biochem. Z.* **310**, 160-169.

284. Spies, T. D. 1946. *South. Med. J.* **39**, 364.
285. Spink, W. W., Wright, L. D., Vivino, J. J., and Skeggs, H. R. 1944. *J. Exptl. Med.* **79**, 331-339.
286. Steinberg, R. A. 1935. *J. Agr. Research* **51**, 413-424.
287. Steinberg, R. A. 1938. *J. Agr. Research* **57**, 261-276.
288. Steinberg, R. A. 1939. *Botan. Rev.* **5**, 327-350.
289. Stiles, H. R., U. S. Patent application No. 434,901, filed March 12, 1942. Quoted by Rudert, F. J. (1945).
290. Stiller, E. T., Keresztesy, J. C., and Stevens, J. R. 1939. *J. Am. Chem. Soc.* **61**, 1237-1242.
291. Stokes, J. L. 1944. *J. Bact.* **47**, 433-434.
292. Stokes, J. L. 1947. *Biol. Symposia* **12**, 227-239.
293. Stokes, J. L., Foster, J. W., and Woodward, C. R., Jr. 1943. *Arch. Biochem.* **2**, 235-245.
294. Stokes, J. L., and Gunness, M. 1945. *J. Biol. Chem.* **167**, 121-126.
295. Stokes, J. L., and Larsen, A. 1945. *J. Bact.* **50**, 219-227.
296. Stokes, J. L., Larsen, A., and Gunness, M. 1947a. *J. Bact.* **54**, 219-230.
297. Stokes, J. L., Larsen, A., and Gunness, M. 1947b. *J. Biol. Chem.* **163**, 613-614.
298. Stubbs, J. J., Noble, W. M., and Lewis, J. C. 1944. *Food Industries* **16**, 694.
299. Sullivan, R. A., Bloom, E., and Jarnol, J. 1943. *J. Nutrition* **25**, 463-470.
300. Swaminathan, M. 1942. *Indian J. Med. Research* **30**, 403-407.
301. Tanner, F. W., Jr., Pfeiffer, S. E., and Van Lanen, J. M. 1945. *Arch. Biochem.* **8**, 29-37.
302. Tanner, F. W., Jr., and Van Lanen, J. M. 1947a. *J. Bact.* **54**, 38-39.
303. Tanner, F. W., Jr., and Van Lanen, J. M. 1947b. U. S. Patent 2,424,003.
304. Tanner, F. W., Jr., Vojnovich, C., and Van Lanen, J. M. 1945. *Science* **101**, 180-181.
305. Tanret, C. 1889. *Compt. rend.* **108**, 98.
306. Tanret, C. 1890. *Ann. chim. physique* **20**, 289.
307. Tatum, E. L. 1944. *Ann. Rev. Biochem.* **13**, 667-704.
308. Tatum, E. L. 1945. *J. Biol. Chem.* **160**, 455-459.
309. Tatum, E. L., and Beadle, G. W. 1942. *Proc. Natl. Acad. Sci. U.S.* **28**, 234-243.
310. Tatum, E. L., Wood, H. G., and Peterson, W. H. 1936. *Biochem. J.* **30**, 1898-1904.
311. Terroine, E. F., Bonnet, R., Knopp, G., and Vechet, J. 1927. *Bull. Soc. chim. biol.* **9**, 678.
312. Teyssie, Y. 1945. *Compt. rend.* **139**, 822.
313. Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A. 1939. *Proc. Soc. Exptl. Biol. Med.* **41**, 194-197.
314. Thaysen, A. C. 1943. *Nature* **151**, 406-408.
315. Thompson, R. C. 1942. University of Texas Bull. No. 4237, 87-97.
316. Tittsler, R. P., and Whittier, F. O. 1941. *J. Bact.* **42**, 151.
317. Totter, J. R. 1946. *Ann. New York Acad. Sci.* **48**, 309-310.
318. Trufanov, A. V., and Ennatskaya, V. V. 1944. *Pishchevaya Prom.* No. 12, 24-30.
319. Underkoffler, L. A., Bantz, A. C., and Peterson, W. H. 1943. *J. Bact.* **45**, 183-190.
320. Van Lanen, J. M. 1946. *Arch. Biochem.* **12**, 101-111.
321. Van Lanen, J. M. Unpublished data, University of Wisconsin, quoted by Massock (1943).

322. Van Lanen, J. M., Broquist, H. P., Johnson, M. J., Baldwin, I. L., and Peterson, W. H. 1942. *Ind. Eng. Chem.* **34**, 1244-1247.
323. du Vigneaud, V., Dittmer, K., Hague, E., and Long, E. B. 1942a. *Science* **96**, 186.
324. du Vigneaud, V., Dittmer, K., Hofmann, K., and Melville, D. B. 1942b. *Proc. Soc. Exptl. Biol. Med.* **50**, 374-375.
325. du Vigneaud, V., Hofmann, K., Melville, D. B., and Gyorgy, P. 1941. *J. Biol. Chem.* **140**, 643-651.
326. Vinson, L. J., Cerecedo, L. R., Mull, R. P., and Nord, F. F. 1945. *Science* **101**, 388-389.
327. Waisman, H. A., Mickelsen, O., McKibbin, J. M., and Elvehjem, C. A. 1940. *J. Nutrition* **19**, 483-492.
328. Waller, C. W., Hutchings, B. L., Mowat, J. H., Stokstad, E. L. R., Boothe, J. H., Angier, R. B., Semb, J., SubbaRow, Y., Cosulich, D. B., Fahrenback, M. J., Hulquist, M. E., Kuh, E., Northey, E. H., Seeger, D. R., Sickels, J. P., and Smith, J. M., Jr. 1946. *Ann. New York Acad. Sci.* **48**, 283-287.
329. Walton, M. T. 1945. U. S. Patent 2,368,074.
- 329a. Waring, W. S., and Werkman, C. H. 1943. *Arch. Biochem.* **1**, 303-310.
330. Weiland, P., and Prelog, V. 1947. *Helv. Chim. Acta.* **30**, 1028-1030.
331. Weinstock, H. H., Jr., Mitchell, H. K., Pratt, E. F., and Williams, R. J. 1939. *J. Am. Chem. Soc.* **61**, 1421-1425.
332. Wenck, P. R., Peterson, W. H., and Fred, E. B. 1935. *Zentr. Bakt. Parasitenk.* **II**, **92**, 330-338.
333. Wenck, P. R., Peterson, W. H., and Greene, H. C. *Zentr. Bakt. Parasitenk.* **II**, **92**, 324-330.
334. West, P. M., and Wilson, P. W. 1938. *Nature* **142**, 397-398.
335. Westenbrink, H. G. K., and Veldman, H. 1942. *Enzymologia* **10**, 255-256.
336. Weyer, E. R., and Rettger, L. F. 1927. *J. Bact.* **14**, 399-424.
337. Wickerham, L. G., Flickinger, M. H., and Johnson, R. M. 1946. *Arch. Biochem.* **9**, 95-98.
338. Wieland, T., and Moller, E. F. 1941. *Z. physiol. Chem.* **269**, 227-235.
339. Wieland, T., and Moller, E. F. 1942. *Z. physiol. Chem.* **272**, 232-238.
340. Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D. 1933. *J. Am. Chem. Soc.* **55**, 2912-2927.
341. Williams, R. J., and Rohrmann, E. 1936. *J. Am. Chem. Soc.* **58**, 695.
342. Winnick, T., Hofmann, K., Pilgrim, F. J., and Axelrod, A. E. 1945. *J. Biol. Chem.* **161**, 405-410.
343. Wirth, J. C., and Nord, F. F. 1942. *Arch. Biochem.* **1**, 143-163.
- 343a. Wolf, D. E., Anderson, R. C., Kaczka, E. A., Harris, S. A., Arth, G. E., Southwick, P. L., Mozingo, R., and Folkers, K. 1947. *J. Am. Chem. Soc.* **69**, 2753-2759.
344. Woods, D. D. 1940. *Brit. J. Exptl. Path.* **21**, 74-89.
345. Woods, D. D. 1947. *Ann. Rev. Biochem.* **16**, 605-630.
346. Woolley, D. W. 1939. *J. Biol. Chem.* **130**, 417-419.
347. Woolley, D. W. 1941. *J. Biol. Chem.* **140**, 453-459.
348. Woolley, D. W. 1945. *Proc. Soc. Exptl. Biol. Med.* **60**, 225-228.
349. Woolley, D. W., and McCarter, J. R. 1940. *Proc. Soc. Exptl. Biol. Med.* **45**, 357-360.
350. Woolley, D. W., and White, A. G. C. 1943. *J. Exptl. Med.* **78**, 489-497.
351. Wright, L. D., and Skeggs, H. R. 1944. *Proc. Soc. Exptl. Biol. Med.* **55**, 92-95.
352. Wright, L. D., Skeggs, H. R., and Welch, A. D. 1944. *Federation Proc.* **3**, 88.

- 353. Yamasaki, I. 1939. *Biochem. Z.* **300**, 160-166.
- 354. Yamasaki, I. 1943. *J. Agr. Chem. Soc. Japan* **16**, 169, 1940; *Biol. Abstracts* **17**, 24502.
- 355. Yamasaki, I. 1941. *Biochem. Z.* **307**, 431-441.
- 356. Yamasaki, I. 1942. U. S. Patent 2,297,671.
- 357. Yamasaki, I., and Morista, S. 1941. *J. Agr. Chem. Soc. Japan* **17**, 86.
- 358. Yamasaki, I., and Yoshitome, W. 1938. *Biochem. Z.* **297**, 398-441.
- 359. Zirplel, W. 1941. *Z. Botan.* **36**, 538-561.
- 360. Zook, H. D., Oakwood, T. S., and Whitmore, F. C. 1944. *Science* **99**, 427-428.
- 361. Zorkoczy, J. 1942. *Cong. intern. tech. chim. ind. agr., Compt. rend. III. Cong. Budapest*, **3**, 10-19, 1943; *Chem. Abstract* **36**, 5850.

The B Vitamins as Plant Hormones

By JAMES BONNER AND HARRIET BONNER

*William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of
Technology, Pasadena, California*

CONTENTS

	<i>Page</i>
I. Introduction.....	225
II. B Vitamins as Root Growth Factors.....	226
1. Requirements of Isolated Roots.....	226
2. Specificity of Thiamine.....	230
3. Specificity of Niacin.....	234
4. Specificity of Pyridoxine.....	235
5. Deficiency Symptoms.....	235
6. Relation of Isolated Roots to Other B Vitamins.....	236
III. B Vitamins as Embryo Growth Factors.....	237
1. Culture of Isolated Embryos.....	237
2. Cultures of Callus and Cambium.....	239
IV. Leaf Growth Factors.....	239
V. Stem Growth Factors.....	241
VI. Distribution of B Vitamins in the Plant.....	241
1. Vegetative Plant.....	241
2. Flowers, Fruits, and Seeds.....	245
VII. Translocation of the B Vitamins.....	247
1. Vegetative Plants.....	247
2. Translocation of Vitamins into Reproductive Organs.....	250
3. Behavior of B Vitamins in Seeds during Germination.....	251
VIII. B Vitamins as Growth Factors for Intact Plants.....	254
IX. Vitamins of the B Complex as Growth Factors for Cuttings.....	259
X. Biochemistry and Metabolism of the B Vitamins.....	260
1. Thiamine.....	260
2. Riboflavin.....	262
3. Niacin.....	263
4. Pyridoxine.....	264
5. Pantothenic Acid.....	265
6. Biotin.....	266
7. Adenine and Other Purines.....	267
XI. Summary.....	268
References.....	270

I. INTRODUCTION

It has been known for many years that vitamins of the B group occur richly in the higher plants, in leaves, seeds, and in other organs. The

discovery in 1934 by Schopfer and by Burgeff that one member of the B group, thiamine, is an essential growth factor for a particular fungus initiated a mass of work by many investigators which has resulted in our present clear picture of the functioning of the vitamins in regulation of the growth of microorganisms. Similarly with the demonstration that riboflavin is a component of an enzyme (Warburg and Christian, 1932) the biochemical role of vitamins in a wide variety of organisms has gradually unfolded. With regard to the functioning of vitamins of the B group in the higher plants, however, a more complex situation exists than in the microorganisms or in the higher animals. Unlike the latter, the higher plant is in general able to synthesize its own vitamins, so that elucidation of the part played by vitamins by simple depletion experiments is not possible. A partial understanding of vitamin economy in the higher plant and of the basic and general role of vitamins in the plant has been made possible only by application of plant tissue culture techniques to the problem. These studies have revealed that certain of the B vitamins function in the plant as hormones, synthesized in one portion of the plant and translocated to other portions where they are needed in growth and development. Thus while the plant as a whole is autotrophic with respect to the B vitamins, the individual organs taken separately differ widely in their ability to synthesize particular members of the B complex. This review will summarize the present knowledge concerning the hormonal role of the B vitamins in the higher plant and will, it is hoped, by indicating the points at which our present information is deficient, stimulate further investigation in this field. The substances to be considered in this review include thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, *p*-aminobenzoic acid, and the purines, especially adenine. So little is known concerning the physiological roles of folic acid, inositol, and choline in the higher plant as to make discussion of them at present unprofitable.

II. B VITAMINS AS ROOT GROWTH FACTORS

1. Requirements of Isolated Roots

It is possible to grow pure cultures of the isolated roots of higher plants provided that the nutrient medium in which they are placed supplies not only those materials which the root normally obtains from the soil but also those materials with which the root normally is supplied by the aerial portions of the plant. The general technique used in isolated root culture is to germinate seeds aseptically, and, when the seedling roots have attained a length of one to several centimeters, to excise the growing

tips and transfer them to nutrient solution contained in flasks or Petri dishes. If an appropriate nutrient solution has been selected the tips continue to grow at approximately the rate obtaining for roots attached to the intact plant. After a period of one to several weeks, the growing tip or tips of the cultured roots may be again excised and transferred to fresh medium. This procedure may be continued indefinitely in the appropriate nutrient, and White (1934) has in fact maintained isolated tomato roots in culture for upwards of 490 passages. With some species, as the tomato (White, 1934), each root makes numerous branches which may be cultured so that clones representing individual original plants may be built up. With other roots, as the pea (Bonner and Addicott, 1937), the branch roots cannot be cultured as normal roots and it is necessary to continue in culture only the original seedling root.

The culture of isolated roots was initiated by Robbins in 1922. Robbins, who used primarily roots of corn, and to a lesser extent pea, sunflower, and lupine, found that although in most cases the freshly excised roots grew vigorously when placed in culture in medium containing mineral salts and sugar, they nevertheless grew less in each succeeding transfer until growth ceased altogether. He suggested that "the failure of an excised root to continue growth when repeated transfers of the root tip are made at once suggests that the seedling root contains some material derived from the seed other than glucose, the mineral salts . . . , water and free oxygen which are necessary for continued growth and which cannot be synthesized in the dark in solution cultures from the material supplied." Various extracts of seeds, seedlings, plants, peptone, and yeast were not able to supply the missing factor in his experiments (Robbins, 1922b). Robbins therefore early noted the possibility that the root may obtain specific root growth factors from other portions of the plant. It is of interest to note that to this day corn roots cannot be grown in culture indefinitely and that the nature of the growth factors required by corn is still obscure.

Material progress in the study of isolated root culture was announced by P. R. White in 1934. White had the good fortune to select tomato roots for his later experiments and he showed that these may be grown indefinitely in culture in a medium containing sugar, mineral salts and 0.01% of yeast extract. The yeast extract was essential to continued growth. The nature of the growth factors contained in yeast extract and essential to growth of isolated roots was next investigated by Bonner (1937) and by Robbins and Bartley (1937a) with pea and tomato respectively. Pea roots, like tomato, could be grown indefinitely in nutrient solution containing sugar, mineral salts, and yeast extract. The yeast extract could, however, be completely replaced for several passages by

crystalline thiamine in a concentration of 0.1 mg./l. nutrient. Robbins and Bartley (1937a) similarly showed that yeast extract may be partially replaced by thiamine in the culture of tomato roots. The importance of thiamine as a growth factor for isolated tomato roots was also recognized by White (1937). Thiamine is, then, one factor required by isolated roots for their growth. It is not, however, the only factor. Isolated pea roots grown on media supplemented with thiamine alone ultimately decrease in growth rate and grow progressively thinner. The second factor required by pea roots was shown by Addicott and Bonner (1938) (see also Addicott and Devirian, 1939) to be niacin. In the presence of thiamine and niacin (0.5 mg./l.) pea roots were maintained through 52 weekly transfers with no diminution in rate. Tomato roots, on the other hand, require primarily pyridoxine in addition to thiamine (Robbins and Schmidt, 1939a, 1939b, 1939c) and with these two substances combined as growth factors tomato roots can be grown indefinitely in culture.

Thiamine, niacin, and pyridoxine are required as growth factors in the cultivation of a wide variety of isolated roots in addition to pea and tomato, and the presently available information is summarized in Table I. One species, flax, has been found which is able to make a limited growth through an indefinite number of transfers in the absence of any added growth substance. The growth of these roots is, however, increased more than 10 times by the addition of thiamine to the medium. Flax further requires only thiamine for indefinite rapid growth. White clover, like flax, is able to grow indefinitely at a slow rate in the absence of thiamine but requires niacin. Of the other 12 species which have been investigated as to requirements for growth *in vitro*, all require thiamine. Seven species including pea, clover, alfalfa, cotton, *Crepis*, *Cosmos*, and radish of the varieties tested are able to grow indefinitely on media containing thiamine and niacin as the sole added growth factors. The further addition of pyridoxine to these roots is without effect. Two species, carrot and some clones of tomato, grow at a maximum rate in the presence of thiamine plus pyridoxine, the addition of niacin being without effect on growth rate. It has been reported by Robbins and Schmidt (1939a) that at least one strain of tomato roots is able to grow indefinitely, although at an exceedingly low rate, in the presence of thiamine as the sole added growth factor, but in any case growth is greatly increased by addition of pyridoxine. Four of the species investigated require thiamine and pyridoxine as growth factors but are caused to make still faster growth by the addition of niacin. These include sunflower, some strains of tomato, *Acacia*, and *Lycopersicon pimpinellifolium*. Of the 14 species investigated only one, *Datura Stramonium*, requires all three growth factors for indefinite growth in culture.

In all of the 14 cases given in Table I, additions of further substances, such as other vitamins of the B complex, purines, pyrimidines, ascorbic acid, amino acids etc., are without effect on growth rate. In addition the combination of vitamins listed for each root elicits in every case even

TABLE I
Growth Factor Requirements of Various Species of Isolated Roots

Species	Common name	Requirement for:			Reference
		Thia- mine	Niacin	Pyrid- oxine	
<i>Linum usitatissimum</i>	Flax	Stim. ¹	None	None	Bonner and Devirian, 1939
<i>Raphanus sativus</i>	Radish	+ ²	+	None	Bonner and Devirian, 1939
<i>Medicago sativa</i>	Alfalfa	+	+	None	Bonner, 1940e
<i>Trifolium repens</i>	White clover	Stim.	+	None	Bonner, 1940e
<i>Gossypium hirsutum</i>	Cotton	+	+	None	Bonner, 1940e
<i>Crepis rubra</i>	Crepis	+	+	None	Miller, 1940
<i>Cosmos sulphureus</i>	Cosmos	+	+	None	Bonner, 1941
<i>Pisum sativum</i>	Pea	+	+	None	Addicott and Bonner, 1938
<i>Daucus Carota</i>	Carrot	+	None	+	Bonner, 1940e
<i>Lycopersicon esculentum</i>	Tomato ³	+	None	+	Robbins and Schmidt, 1939a, b. Bonner, 1943a
<i>Lycopersicon esculentum</i>	Tomato ³	+	Stim.	+	Bonner and Devirian, 1939 Robbins, 1941
<i>Lycopersicon pimpinellifolium</i>		+	Stim.	+	Bonner, 1940e Robbins, 1941
<i>Helianthus annuus</i>	Sunflower	+	Stim.	+	Bonner, 1940e
<i>Acacia melanoxylon</i>	Acacia	+	Stim.	+	Bonner, 1942
<i>Datura Stramonium</i>	Jimson weed	+	+	+	Bonner, 1940e

¹ Factor not essential but does increase root growth.

² Factor essential for root development.

³ Refers to different clones of tomato roots.

faster growth than that elicited by the optimum concentration of yeast extract. It is unlikely, therefore, that other unrecognized growth factors are needed by roots of these particular species for optimal growth *in vitro*. A possible exception to this rule is the case of the amino acid glycine, which was at one time reported by White (1939b) to replace pyridoxine as a growth factor for tomato roots. In a later paper White (1943) has, however, come to the view that glycine cannot replace pyridoxine, but may at most increase growth somewhat in the presence of thiamine, pyridoxine, and niacin. Other investigators (Bonner, 1943a; Robbins and Schmidt, 1939b) have failed to find any influence of

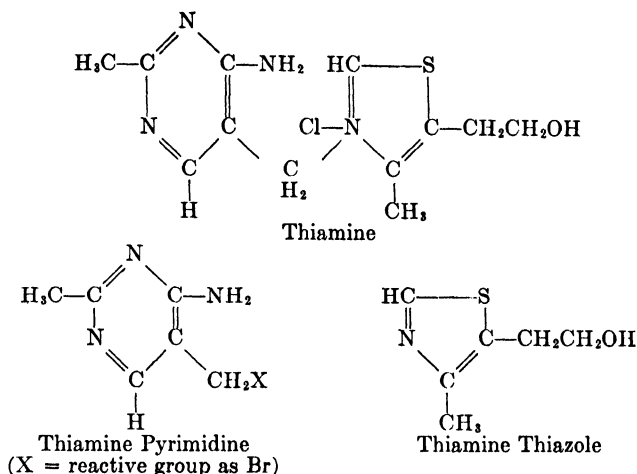
glycine on the growth of isolated tomato roots either in the presence or absence of pyridoxine.

The results presented in Table I should not convey the impression that any and all species of roots may be readily cultivated *in vitro* in a purely synthetic medium. On the contrary, the number of species whose culture *in vitro* has been unsuccessfully attempted exceeds the number for which such attempts have been successful. The roots of the *Gramineae* form a group which have resisted indefinite culture on any medium. Thus the early unsuccessful attempts of Robbins (1922a, 1922b) with corn roots were followed by unsuccessful attempts with the same plant by Fiedler (1936), by White (1932a) with roots of wheat and by the present author with roots of corn, wheat, rice, *Poa* species as well as a variety of other monocotylenous plants (Bonner, 1940a). In all cases the roots grow well in culture immediately after excision but decrease in growth with each successive transfer, indicating that growth factors present in the seedling root are being depleted during transfer. It has not proved possible to supply this missing factor from any crude source such as extracts of yeast, seeds, seedlings, or leaves, or by any known substance or combination of substances. A report of McClary (1940) that corn roots were grown successfully through 18 transfers on a medium containing only sucrose, agar, and salts has not been confirmed (Bonner 1940a). Roots of woody species in general exhibit behavior similar to that of the grasses and to date only one woody species, *Acacia melanoxylon*, has been successfully maintained in culture (Bonner, 1942c). Not all species of dicotylenous herbaceous plants which have been attempted have yielded to culture techniques. For example, no cucurbit root has been grown on a known nutrient medium.

In summary, the isolated roots of a number of plant species have been cultured *in vitro* through repeated transfers on medium containing only known substances. All of these species of isolated roots require thiamine for maximum growth in culture and all but one require additionally either pyridoxine or niacin or both.

2. Specificity of Thiamine

It has been shown by Robbins and Bartley (1937b) for the tomato root and by Bonner (1938a) for the pea root that in these cases the intact thiamine molecule can be quantitatively replaced as a growth factor by an equimolecular mixture of two fragments of the thiamine molecule, representing the thiazole and pyrimidine portions respectively. The two fragments, whose structure is shown on page 231, are combined in the plant into thiamine itself (Bonner and Buchman, 1938). In individual strains of tomato roots, thiamine may be replaced by the vitamin thiazole

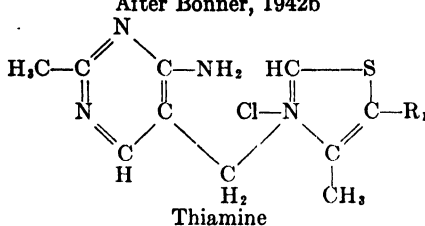


alone, addition of the pyrimidine being unnecessary (Robbins and Bartley, 1937b). It is presumed that in this case the root possesses the ability to synthesize the vitamin pyrimidine so that the compound is available for combination with added thiazole. Other strains of tomato roots, like the pea, require that both the thiazole and pyrimidine fragments of the vitamin be added to the medium (Bonner and Devirian, 1939). Flax, like the tomato strain of Robbins, requires only the thiazole fragment rather than the intact thiamine molecule (Bonner, 1940b).

The structural specificity of thiamine as a root-growth factor has been investigated only to a limited extent so far as analogues of the intact thiamine molecule are concerned. Table II summarizes the data available for excised pea roots. Analogs in which the 5- α -hydroxyethyl group is replaced by β -hydroxyethyl or hydroxypropyl side chains are not able to support continued root growth. This is true even at levels 10 times those in which thiamine is used. Similarly pyamine analogues of thiamine are ineffective. Comparable results have also been obtained with isolated tomato roots (Bonner, 1940c). A greater range of structures has been investigated using combinations of thiazole analogues with the vitamin pyrimidine or of pyrimidine analogues with the vitamin thiazole. Table III gives a summary of certain thiazole analogs tested for ability to support the growth of pea roots in the presence of the vitamin pyrimidine and of niacin. Two of the analogues, in which a β -hydroxy- or a γ -hydroxypropyl group is substituted for the α -hydroxyethyl group of the vitamin thiazole appear to be able to support growth of pea roots over considerable periods of time, although at a lesser rate than the vitamin thiazole itself given in the same concentration. This is in contrast to the behavior of the vitamin analogues containing the same two

TABLE II

*The Structural Specificity of Thiamine as a Root Growth Factor for Isolated Pea Roots.
All Roots Grown in a Medium Containing Inorganic Salts, Sucrose, and Niacin
After Bonner, 1942b*



Analogue	R ₁ (Thiazole ring)	Effect on growth
γ-Hydroxypropyl vitamin analogue...	—CH ₂ —CH ₂ —CH ₂ OH	No growth after four transfers
β-Hydroxypropyl vitamin analogue...	—CH ₂ —CHOH—CH ₃	No growth after four transfers
α-Hydroxyethyl vitamin analogue...	—CHOH—CH ₃	No growth after four transfers
β-Hydroxyethyl (thiamine).....	—CH ₂ —CH ₂ OH	Indefinite growth at an average of 90 mm./week

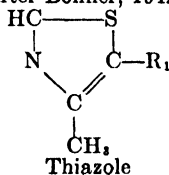
thiazoles since the vitamin analogues, as shown in Table II, are inactive. This remarkable difference between the activity of the two analogues when given as free thiazoles and when combined into a vitamin analogue is not found in other organisms where they have been tested. Thus, in *Phycomyces*, both forms of the analogues are inactive or essentially so (Bonner, 1940c). A possible explanation might be found in a thiazole sparing action of the free thiazole analogues, the analogues in some way acting to conserve the thiazole already contained in the root tip. An analogue of particular interest is β-4-methylthiazole alanine, an amino acid synthesized by Buchman and Richardson (1939), containing a 4-methylthiazole linked through the 5 position to the β carbon atom of alanine. This amino acid is active as a source of thiazole both in the pea root (Bonner and Buchman, 1938) and in the tomato (Robbins, 1940). It has been shown in the pea root that the compound is transformed *in vivo* into the vitamin thiazole (Bonner and Buchman, 1938). Although this fact shows that enzymes for the transformation of the amino acid must occur in the plant, still the amino acid is as yet unknown in nature and we have no knowledge as to any possible role which it might play.

Earlier work on the specificity of various thiazole analogues in replacing the vitamin thiazole as a growth factor for isolated pea roots (Bonner,

TABLE III

Structural Specificity of the Thiazole Portion of the Thiamine Molecule as a Growth Factor for Isolated Pea Roots. All Roots Grown in a Medium Containing Inorganic Salts, Sucrose, Niacin, and Vitamin Pyrimidine

After Bonner, 1942b



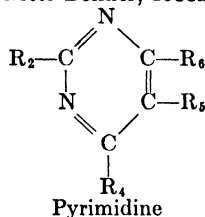
Thiazole analogue	R ₁	Effect on growth
Vitamin thiazole.....	—CH ₂ CH ₂ OH	Indefinite growth at average of 90 mm./week
α-Hydroxyethyl vitamin thiazole..	—CHOHCH ₃	No growth after four transfers
γ-Hydroxypropyl vitamin thiazole	—CH ₂ CH ₂ CH ₂ OH	Growth continued through six transfers at an average of 55 mm./week
β-Hydroxypropyl vitamin thiazole	—CH ₂ CHOHCH ₃	Growth continued through twelve transfers at an average of 48 mm./week
Vitamin thiazole alanine.....	—CH ₂ CHNH ₂ COOH	Indefinite growth at rate equal to vitamin thiazole
Imidazole analogue.....	No growth after four transfers
Vitamin thiazole-5-carboxylic acid	—COOH	No growth after four transfers

1938a) has indicated a marked lack of specificity of the thiazole portion. In contrast to the data given in Table III the earlier work was carried out with roots grown in a medium lacking niacin and hence not growing at a maximal rate. The low specificity, which contrasts strongly with the great specificity found for microorganisms (Bonner and Erickson, 1938; Robbins and Kavanagh, 1938b; Schopfer, 1934; Knight, 1937a, b; Tatum and Bell, 1946) may again be due to thiazole sparing action or other secondary effects.

The small amount of work which has been reported on the structural specificity of the pyrimidine portion of thiamine as a root growth factor indicates that even small changes in this ring abolish activity altogether. This is shown in Table IV, which refers to pea roots grown on a medium containing salts, sucrose, and vitamin thiazole. Analogues in which the

TABLE IV

Structural Specificity of the Pyrimidine Portion of the Thiamine Molecule as a Growth Factor for Isolated Pea Roots. All Roots Grown in Medium Containing Salts, Sucrose, and Vitamin Thiazole
After Bonner, 1938a



Number	R ₂	R ₄	R ₅	R ₆	Activity
1	CH ₃	H	CH ₂ Br	NH ₂	Active
2	CH ₃	H	CH ₂ NH ₂	NH ₂	Active
3	CH ₃	H	CH ₂ OEt	NH ₂	Active
4	CH ₃	H	CH ₂ NHCSH	NH ₂	Active
5	CH ₃	H	H	NH ₂	Inactive
6	CH ₃	H	CH ₂ COOH	NH ₂	Inactive
7	CH ₃	H	CH ₂ CONH ₂	NH ₂	Inactive
8	CH ₃	H	CH ₂ OEt	OH	Inactive
9	CH ₃	H	CH ₂ NH ₂	OH	Inactive
10	CH ₃	H	CH ₂ OH	OH	Inactive
11	OH	CH ₃	CH ₂ OH	OH	Inactive
12	Cl	CH ₃	CH ₂ thiazole	NH ₂	Inactive

methylene group of the 5 position is substituted with Br, NH₂, or substituted amino groups are active. The substitution of OH for NH₂ at position 6 abolishes activity altogether. These results are concordant with those obtained for other organisms (Bonner and Erickson, 1938; Robbins and Kavanagh, 1938a), although it should be noted that with *Phycomyces*, replacement of the 2-methyl by an ethyl group leaves the activity of the pyrimidine substantially unaltered (Robbins and Kavanagh, 1938c).

3. Specificity of Niacin

The specificity of niacin as a growth factor for isolated pea roots has been investigated (Bonner, 1940d) and a summary of the results is given in Table V. Niacinamide is as active as niacin, as are also derivatives which may be readily convertible into niacin, including esters of the acid and nicotinuric acid. All other compounds tested were inactive. The specificity of niacin as a growth factor for pea roots hence parallels closely the results obtained with *Staphylococcus aureus* (Knight, 1937a, b; Landy,

TABLE V

Structural Specificity of Niacin as a Growth Factor for Isolated Pea Roots. All Roots Grown in a Medium Containing Inorganic Salts, Sucrose, and Thiamine After Bonner, 1940d

Substance	Activity
Niacin.....	Fully active
Niacinamide.....	Fully active
Coramine (<i>N</i> -diethylamide of niacin).....	Inactive
Methiodide of niacinamide.....	Inactive
Methyl nicotinate.....	Fully active
Ethyl nicotinate.....	Fully active
Propyl nicotinate.....	Fully active
Butyl nicotinate.....	Fully active
Nicotinuric acid.....	Fully active
Isonicotinic acid.....	Inactive
Picolinic acid.....	Inactive
Quinolinic acid.....	Inactive
Dinicotinic acid.....	Inactive
Ethyl nicotinylacetate.....	Inactive
Arecoline.....	Inactive
β -Aminopyridine.....	Inactive
Nicotino-3-nitrile.....	Inactive
β -Picoline.....	Inactive
Thiazole-5-carboxylic acid.....	Inactive
Amide of thiazole-5-carboxylic acid.....	Inactive
Benzoic acid.....	Inactive
Pyrazine-3-carboxylic acid.....	Inactive
Pyrazine-2, 3-dicarboxylic acid.....	Inactive

1938), dysentery bacteria (Dorfman *et al.*, 1938, 1939) and the dog (Woolley *et al.*, 1938).

4. Specificity of Pyridoxine

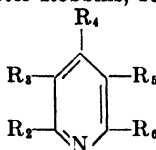
The specificity of pyridoxine as a growth factor for isolated tomato roots has been investigated by Robbins (1942) and a summary of the results obtained is given in Table VI. Aside from compounds readily convertible to pyridoxine only one analogue, i.e., that in which the methyl group at position 2 is replaced by an ethyl group, is active as a growth factor in this case, a result in general agreement with that obtained with the rat (Unna, 1940) and with lactic acid bacteria (Bohonos *et al.*, 1941).

5. Deficiency Symptoms

It is evident from what has been stated above that isolated roots cultured in media deficient in a particular required growth factor exhibit the generalized deficiency symptom of reduced growth in length. More specific deficiency symptoms are, however, evident on detailed inspection. Pea roots grown on a medium containing salts, sucrose, and niacin

TABLE VI

Structural Specificity of Pyridoxine as a Growth Factor for Isolated Tomato Roots. All Roots Grown in a Medium Containing Salts, Sucrose, and Thiamine
After Robbins, 1942



Position 2	3	4	5	6	Activity
CH ₃	OH	CH ₂ OH	CH ₂ OH	H	Active (pyridoxine)
CH ₃	CH ₂ COO	CH ₂ CH ₂ COO	CH ₂ CH ₂ COO	H	Active
CH ₃	OH	CH ₂ CH ₂ COO	CH ₂ CH ₂ COO	H	Active
CH ₃	CH ₃ O	CH ₂ OH	CH ₂ OH	H	Inactive
CH ₃	OH	CH ₃	CH ₃	H	Inactive
CH ₃	NH ₂ ·HCl	CH ₂ OH	CH ₂ NH ₂ ·HCl	H	Inactive
CH ₃	OH	CH ₂ OC ₂ H ₅	CH ₂ OH	H	Inactive
CH ₃	NH ₂ ·HCl	CH ₂ OC ₂ H ₅	CH ₂ NH ₂ ·HCl	H	Inactive
CH ₃	OH	CH ₃	CH ₂ OH	H	Inactive
C ₂ H ₅	OH	CH ₂ OH	CH ₂ OH	H	Active

but deficient in thiamine show greatly reduced meristematic activity. The cells of the meristematic region present before the onset of thiamine deficiency attain essentially the normal size and differentiation into vascular elements occurs normally (Addicott, 1939, 1941). Thiamine deficient roots consist therefore of short sections of mature root but possess only a rudimentary meristem. In niacin deficiency, on the contrary, the roots grow progressively thinner due to reduction of the number of cell columns in the meristem (Addicott, 1941) as well as to reduction in width of the individual cells. The cells of the niacin deficient roots do not elongate normally and attain only approximately 60% of the length of the cells of the normal roots. Meristematic activity is somewhat reduced in the tips of niacin deficient roots, but the reduction is less marked than in the case of thiamine deficiency (Addicott, 1941). In general it may be said that niacin deficiency results primarily in reduction of cell elongation. Pyridoxine deficiency has been histologically investigated in tomato and in *Datura* roots by Addicott (1942). Pyridoxine deficiency, like thiamine deficiency, results in lowered cell division activity in the root meristem.

6. Relation of Isolated Roots to Other B Vitamins

Only three of the vitamins of the B complex are required as constituents of the medium for the growth of isolated roots. The three

vitamins involved are hence evidently those which the roots in question are unable to synthesize. It is of interest to know whether the other vitamins of the B complex are synthesized by roots from the simple constituents of the medium or whether they are not needed in the root economy. It is now evident that the general answer to this question is that isolated roots synthesize those members of the B complex which they do not require as externally supplied growth factors. A simple case is that of flax roots which (as mentioned above) can grow, although at a low rate, in medium consisting only of inorganic salts and sucrose. These roots synthesize thiamine to the extent of *ca.* 0.02 γ /root/week under the culture conditions used (Bonner, 1940c). This amount is small in comparison with the 2 γ /root/week which must be added to the culture medium in order to obtain maximal growth. A similar small synthesis of thiamine was found in the case of clover roots, which, like flax, are able to grow at a low rate in the absence of added thiamine.

Biotin, which is not required as a supplement for the growth of any root yet investigated, is abundantly synthesized by roots of flax, clover, alfalfa, and tomato (Bonner, 1940e). *p*-Aminobenzoic acid is synthesized by isolated tomato roots and, incidentally, inhibition of the growth of isolated tomato roots by sulfa drugs is of a competitive nature, the inhibition by sulfathiazole, for example, being largely reversible by addition to the medium of *p*-aminobenzoic acid (Bonner, 1942f). Riboflavin is likewise synthesized by isolated roots of tomato, clover, alfalfa, *Datura*, and sunflower (Bonner, 1942d). Information on the synthesis of pantothenic acid, inositol, folic acid, and other members of the B complex by roots is not available. It seems, however, most probable that these compounds, like riboflavin, biotin, and *p*-aminobenzoic acid, are required by roots, just as by other tissues and organisms, but that the root cells possess the power of synthesizing the compounds in question from the simple constituents of the medium.

III. B VITAMINS AS EMBRYO GROWTH FACTORS

1. *Culture of Isolated Embryos*

The fact that vitamins of the B complex are stored in high concentrations in the seeds or seed parts of higher plants suggests at once that these vitamins may play a role in the embryo development, seed germination, or early growth of the plant. That this is in fact the case was first suggested by work of Kögl and Haagen-Smit (1936) with excised pea embryos from which the cotyledons had been removed and which were grown in aseptic culture. Under these conditions relatively little growth occurred on a medium containing only mineral salts and sugar. Addition of biotin to the medium resulted in considerable increases in rate of

stem growth while addition of thiamine resulted in increased growth, primarily of roots, but also to a lesser extent of the shoots. These results were extended by Bonner and Axtman (1937) who found that pantothenic acid like biotin increases the growth of stems of cotylectomized pea embryos. Additions of estrone and ascorbic acid also increased growth, although ascorbic acid is extensively synthesized by some varieties of pea embryos (Bonner and Bonner, 1938). Niacin, like thiamine increases the growth of both roots and shoots of cotylectomized pea embryos (Bonner, 1938b). Even with the optimum combination of biotin, thiamine, pantothenic acid, niacin, estrone, and ascorbic acid growth of such embryos does not approach that of plants from which cotyledons have not been removed (Bonner, 1942a) and it is evident that other as yet unknown factors are of importance in the growth of pea embryos.

The culture of immature plant embryos has long attracted attention, but detailed study of the nutritional requirements of such embryos has been made only by Van Overbeek *et al.* (1941) in the case of *Datura Stramonium*. Mature or nearly mature embryos were grown successfully in medium containing dextrose and mineral salts alone, such embryos requiring no added growth factors for further development. Embryos which were only one-third of the final size did not develop in the simple medium, however, and were only able to develop if, in addition to salts and sugar, a complex mixture of vitamins and other possibly active substances was present. This mixture included glycine, thiamine, ascorbic acid, niacin, pyridoxine, adenine, succinic acid, and pantothenic acid. The active constituents of the mixture were identified by Loo (1944) who found only pantothenic and succinic acids to be essential, the other substances being without effect. Embryos smaller than one-third of the final size were found by Van Overbeek *et al.* (1941) to develop only in still more complex media containing unknown substances derived from coconut milk. Immature embryos of other species have been grown in media containing yeast extract or fibrin digest (LaRue, 1936; White, 1932b; Brink *et al.*, 1944; Tukey, 1934).

Only a small beginning has been made in the study of the growth factor requirements of embryos during their development. It is clear that mature embryos are able to develop in simple media without additional growth factors although in some cases, as that of cotylectomized pea embryos, addition of thiamine, niacin, pantothenic acid, and biotin as well as other substances may accelerate growth. In embryos which are only moderately immature such as those of *Datura* (above) pantothenic acid is an essential factor for development. Whether the same substance is limiting in other cases is unknown. In still more immature

embryos, development *in vitro* proceeds only at the expense of other as yet unknown substances which are apparently not members of the B complex. Both the vitamins such as pantothenic acid and the unknown growth factors for immature embryos would appear to constitute specific substances required for embryo development which, since they are not synthesized in the embryo, must move into the embryo from surrounding plant tissues. These materials may properly be referred to as hormones of embryo development.

2. Cultures of Callus and Cambium

Much attention has been paid in recent years to the culture *in vitro* of callus tissue, a subject developed and recently reviewed by Gautheret (1946) and to which White (1939a) has contributed largely. Such callus tissue may be grown *in vitro* indefinitely, the nature of the medium required depending on the tissue involved. Thus the callus tissue cultured from the spontaneous tumors of a *Nicotiana* hybrid (White, 1939a) requires no added growth factors of any kind and is apparently autonomous with respect to the B vitamins (Hildebrandt *et al.*, 1946). Callus tissues induced in normal plants by application of high concentrations of auxin require the presence of indoleacetic acid for continued development *in vitro* (Gautheret, 1946), and Morel (1946) has shown that in one case at least, *Crataegus monogyna*, pantothenic acid is likewise an essential nutrient factor. Sections of the fleshy root of carrot have been cultured by Nobécourt (1943). In contrast to the behavior of seedling carrot roots which require thiamine, the tissues of the fleshy root continue to synthesize thiamine in culture (Nobécourt, 1940). Callus of rose tissue, also cultured by Nobécourt (Nobécourt and Kofler, 1945) is reported to require addition of thiamine for maximum growth.

Other work on callus cultures which need not be reviewed here has shown that indoleacetic acid is of primary importance as a growth factor for many kinds of callus tissue (Gautheret, 1946) and has indicated that tumor tissues may differ from corresponding normal tissue in requirement for this growth factor (Skoog, 1944; DeRopp, 1947). The work of Morel and of Nobécourt does indicate, however, that (in certain cases at least) vitamins of the B complex may be required by callus tissue and it is evident that the possibility of such requirements should be considered in further work.

IV. LEAF GROWTH FACTORS

The growth of the leaf may be divided into two physiologically separate aspects, i.e., growth of the veins and growth of the mesophyll between the veins. Avery (1935) has made it probable that vein growth

in *Nicotiana* is limited by available auxin. Growth of the mesophyll on the contrary involves other growth factors including in particular the purines. Early work of Vyvyan (1924) and Went (1938a, 1938b) has shown that growth of the mesophyll of the leaves of seedlings is dependent on substances stored in the cotyledons while Gregory (1928) has produced evidence that increase in leaf area of young leaves is dependent on the already existing leaf area of the plant. While both of these facts indicate the regulation of leaf development by substances which travel within the plant, they do not indicate whether the substances involved are hormonal in nature or are merely general food materials. D. Bonner *et al.* (1939) have devised an assay for leaf-growth factors based on the increase in area of sections cut from the immature leaves of seedling radish plants. These sections are floated on a solution containing sucrose and the increase in area over a 24-hour period is measured. Extracts of pea seeds (D. Bonner *et al.*, 1939) or extracts of mature leaves (D. Bonner, 1940) when added to the nutrient solution were active in increasing the rate of expansion of such test sections. A second test for leaf-growth factor activity consisted in the aseptic culture in the dark of excised leaf primordia (approximately 1 mm. in length) of pea seedlings in solutions containing salts and sucrose. The addition of pea cotyledon (D. Bonner *et al.*, 1939) or leaf extracts (D. Bonner, 1940) greatly increased the growth of such leaves. Such cultured leaves actually attained a greater size than would have resulted had the leaf remained on the plant. In both of the assay tissues cell divisions were largely completed before excision of the test tissue and growth under influence of the active materials was largely by cell enlargement. Fractionation of the pea cotyledon extract led to the isolation of a highly active compound, hypoxanthine, and tests of pure compounds revealed that adenine also is active in promoting leaf growth. Other compounds of the B complex were inactive. Certain other compounds active in low concentrations found in the course of the work were dihydroxyphenylalanine, isolated from pea cotyledons and the further amino acids proline, asparagine, valine, glutamic acid, alanine, and leucine. So far as the growth of radish leaves is concerned the activity of pea cotyledon extract would appear to depend on varied factors in addition to the purine hypoxanthine. In the culture of excised pea leaves, however, adenine is capable of quantitatively replacing pea cotyledon extract as a leaf growth factor. Isolated whole buds of rye have been grown in culture by DeRopp (1945) and the growth of the leaves observed. Neither vitamins of the B complex nor adenine or other purines were effective in increasing the growth of leaves of this grass. The factors limiting leaf growth in rye are therefore different from those limiting leaf growth in pea or radish.

It may be concluded that while only a modest start has been made on the subject of the leaf growth factors, nevertheless special factors for control of leaf growth do exist, are made or stored in seeds and mature leaves and are required by immature leaves for expansion of the mesophyll cells. While hypoxanthine and adenine have been recognized as important in the control of leaf growth in radish and in pea it remains to be seen whether these compounds are of general significance as leaf growth hormones.

V. STEM GROWTH FACTORS

The growth of isolated stems in culture has been successfully consummated only by Loo (1945). This author cultivated isolated stem tips of asparagus (*Asparagus officinalis*) in nutrient media containing mineral salts and sugar. On this medium the stems grew actively through repeated transfers for periods of more than 1 year, provided that the cultures were maintained in diffuse light. In the dark on the contrary, growth approached zero after four transfers, owing apparently to depletion of growth factors contained in the original tip. Growth was not maintained in the dark by addition of any of the B vitamins, or by various amino acids, although growth during the first transfer was promoted by addition of succinic acid, ammonium sulfate, or aspartic acid. Excised asparagus stem tips appear, then, to synthesize in the light growth factors as yet unknown and which cannot be synthesized in the dark. The amount of light-induced synthesis must be quantitatively small, since an external supply of sucrose is essential even in light-grown cultures.

The growth of excised shoots of tomato plants in the dark has been studied by Went and D. Bonner (1943). The growth rate of such stems approaches zero after approximately 24 hours in darkness but can be increased by supplying the stems with sucrose fed through the leaves. Immersion of leaves in extracts of pea seeds, of yeast, or of coconut milk gave a further increase in growth rate above the level obtained with sucrose alone. The nature of these stem growth factors is, however, still obscure.

VI. DISTRIBUTION OF B VITAMINS IN THE PLANT

1. Vegetative Plant

It has been shown above that vitamins of the B complex are physiologically active in the plant in that they are limiting factors in the development of isolated roots, embryos, and leaves. In order to establish that these same factors act *in vivo* as hormones or correlative agents in plant

growth it is essential to discover whether they are synthesized in particular restricted areas within the organism and subsequently translocated to the organs or tissues which are unable to synthesize them and in which they are required for growth. This section and the following will consider the vitamin economy of the intact plant; the distribution, site of formation, and translocation of B vitamins in the plant.

It may be stated at the outset that the B vitamins are found in all species and in all organs of higher plants, and a brief selection of examples given in Table VII illustrates this point. Of the eight vitamins examined all were detected in all cases. Thiamine occurs in concentrations of 3-14 γ /g. in leaves, roots, and fruits, and riboflavin is found in amounts of approximately the same order of magnitude as are also pantothenic acid, pyridoxine, and folic acid. Niacin occurs in higher concentration, of the order of 50 γ /g. being a usual figure. Inositol is found in very much higher concentrations, of the order of 1000-8000 γ /g. of dry material, while biotin occurs to the extent of from less than 0.1 to 0.7 γ /g. No general rules concerning the relative proportions or amounts of the several vitamins in different tissues as in leaves, roots, or fruits, are evident.

The distribution of thiamine in tomato plants grown in the greenhouse and approximately 40-cm. tall is given in Table VIII. The highest concentration of thiamine, 19.8 γ /g., is found in the shoot apex and youngest leaves. Each successively older leaf has a smaller concentration of the vitamin and a similar gradient in thiamine concentration is found in the stem. The roots are moderately high in thiamine containing 6.7 γ /g. Although the concentration decreases in each successively lower leaf still the total amount of thiamine increases to a maximum in the eighth leaf owing to the increasing weight of the older leaves. This, as will be shown below, is owing to the fact that thiamine is formed in the expanding and recently matured leaves. The thiamine distribution in maize has been investigated by Burkholder and McVeigh (1940). In this plant, as in tomato, thiamine concentrations are higher in younger than in older leaves. A similar distribution of thiamine in the pea plant has been described by Rytz (1939) and in cotton by Sukhorukov and Filippov (1940). The distribution of thiamine in woody plants is similar to that found in annuals (Burkholder and Snow, 1942) and the thiamine content of a variety of leaves and buds has been tabulated by Burkholder and McVeigh (1945a). The occurrence of thiamine as well as of riboflavin, biotin, pantothenic acid, niacin, and folic acid in oat plants of different ages has been studied by Kohler (1944). In general the vitamins tended to attain a maximum concentration in the plant as a whole at approximately the time of flowering. A vast amount of miscellaneous

TABLE VII
B Vitamin Content of Some Typical Plant Tissues
 After Cheldelin and Williams, 1942

Species	Part	Vitamin content in γ /g. dry weight							
		Thia- mine	Ribo- flavin	Niacin	Panto- thenic acid	Pyri- doxine	Biotin	Inositol	Folic acid
<i>Beta vulgaris</i>	Leaves	14	20	58	14	3.6	0.26	2000	20
<i>Spinacea oleracea</i>	Leaves	5.4	22	50	17	8.1	0.67	2600	23
<i>Beta vulgaris</i>	Roots	1.9	4.5	49	8.2	0.97	0.02	1600	3.4
<i>Daucus Carota</i>	Roots	4.2	4.2	22	21	10.2	0.21	4100	8.2
<i>Brassica oleracea</i>	Roots	3.3	8.1	86	4.6	14	0.26	5800	3.3
<i>Pyrus Malus</i>	Whole fruit	6.6	1.2	5.6	4.1	1.8	0.06	1600	0.55
<i>Lycopersicon esculentum</i>	Whole fruit	10	6.2	78	62	10	0.67	7700	13

TABLE VIII

Distribution of Various Vitamins in Tomato Plants Approximately 40-cm. Tall. Figures on Dry Weight-Basis
After Bonner, 1942e and Bonner and Dorland, 1943a, b

Part	Dry weight of part g./2 plants	Thiamine γ /g.	Pyridoxine ¹ γ /g.	Riboflavin γ /g.	Pantothenic acid γ /g.
Apex.....	0.113	19.8	24.6	33.7	35.7
Leaf 12.....	0.102	16.0	43.4	35.6	23.3
11.....	0.172	14.8	26.9	36.6	17.6
10.....	0.270	13.2	24.4	35.8	19.8
9.....	0.356	11.4	22.7	35.6	16.6
8.....	0.536	10.9	22.4	32.0	15.2
7.....	0.516	9.4	23.6	26.0	17.0
6.....	0.528	8.1	20.3	24.4	14.0
5.....	0.392	7.8	20.0	18.6	11.3
4.....	0.344	7.0	20.0	16.4	14.1
3.....	0.242	5.7	16.1	15.2	14.9
2.....	0.161	4.7	14.8	11.4	
1.....	0.137	4.5	10.5	
Top 10 cm. of stem.....	0.310	10.0	6.2	20.8
Next 10 cm. of stem.....	0.620	4.2	5.7	14.4
Next 10 cm. of stem.....	0.572	3.0	4.2	9.7
Bottom 10 cm. of stem.....	0.662	2.4	2.8	8.6
Roots.....	1.135	6.7	13.9	10.3	29.0

¹ Separate set of plants from those used in other determinations.

information on the thiamine content of plants and plant parts of interest from the standpoint of human food value is available but is in general not informative in the present connection.

The detailed distribution of pyridoxine in the vegetative tomato plant generally parallels that of thiamine, the highest concentration being in the younger leaves and lower concentrations in the older leaves, the highest concentration being 43 γ /g. and the lowest 14 γ /g. (Table VIII, Bonner and Dorland, 1943b).

Riboflavin and pantothenic acid distributions generally parallel that of thiamine in the vegetative tomato plant, although the concentration gradient from younger to older leaves is less striking (Table VIII, Bonner and Dorland, 1943a). Although detailed analysis of the distribution of other members of the B complex in the plant is lacking, still it is known, as discussed above, that the other vitamins as well as *p*-aminobenzoic acid and adenine, occur generally in plant tissues including leaves. Thus Burkholder and McVeigh (1945a) found thiamine, riboflavin,

pyridoxine, niacin, inositol, biotin, pantothenic acid, and folic acid in leaves or buds of all of 18 species investigated while Kohler (1944) found thiamine, riboflavin, biotin, pantothenic acid, niacin, and folic acid in *Avena* plants of all ages studied. In summary, it appears probable that the vitamins of the B complex occur universally in higher plant tissues, and in so far as the matter has been looked into, the vitamins are present in highest concentration in the young leaves of the vegetative plant with a general gradient of decreasing concentration toward the older portions. The same vitamins are found also in the stems and roots.

2. Flowers, Fruits, and Seeds

The floral parts of the plant, like the vegetative parts, possess appreciable contents of B vitamins. Thus Rytz (1939) found thiamine in young buds, calyx, corolla, stamens, and pistil of pea and tulip, as is shown in Table IX, while Cravioto *et al.* (1945) found thiamine, riboflavin, and

TABLE IX
Vitamin Contents of Flowers and Floral Organs of Various Species

Species	Thiamine content, γ /g. dry weight				
	Immature buds	Calyx	Corolla	Stamens	Pistil
<i>Pisum sativum</i> ¹	23	21	14	13	20
<i>Tulipa Gesneriana</i> ¹	4	4	10	5

Species	Thiamine, γ /g. dry flower	B ₂ , γ /g. dry flower	Niacin, γ /g. dry flower
<i>Cucurbita mexicana</i> ²	20.0	25.0	122
<i>Brassica campestris</i> ²	4.6	1.5	73
<i>Agave atrovirens</i> ²	12.2	5.6	23.2
<i>Yucca aloifolia</i> ²	14.0	9.0	58.0

¹ After Rytz, 1939.

² After Cravioto, *et al.*, 1945.

niacin in whole flowers of various species. Thiamine as well as niacin, pantothenic acid, and riboflavin have been found in a variety of pollens by Vivino and Palmer (1944). Detailed analysis of the distribution of vitamins other than thiamine in the flower is lacking but the available data, of which a selection is given in Tables VII and X, indicates that fruits and seeds in general contain all members of the complex. Contrary to what might be expected, seeds do not appear to be generally

higher in vitamin content than the other portions of the fruit when compared on a dry weight basis (Table X).

TABLE X
Vitamin Contents of Some Typical Fruits and Seeds
After Cravioto et al., 1945

Species		Vitamin content, γ /g. dry wt.		
		Thiamine	Riboflavin	Niacin
FRUITS				
<i>Cucurbita mexicana</i>	Squash	10.0	10.0	70
<i>Cucumis sativus</i>	Cucumber	6.5	8.7	39.2
<i>Persea gratissima</i>	Avocado	3.4	4.9	39.4
<i>Prunus Armeniaca</i>	Apricot	1.8	1.8	25.1
<i>Passiflora lingularis</i>	Passion fruit	0.5	4.7	70.2
<i>Lycopersicon esculentum</i>	Tomato	11.1	5.6	66.3
<i>Citrus nobilis</i>	Tangerine	6.6	2.0	14.5
<i>Mangifera indica</i>	Mango	6.9	2.4	80.5
<i>Cucumis Melo</i>	Melon	4.6	3.1	81.6
<i>Citrus Aurantium</i>	Orange	6.7	2.2	18.6
SEEDS				
<i>Sesamum indicum</i>	Sesame	14.2	2.4	52.3
<i>Phaseolus vulgaris</i>	Bean	9.2	2.0	24.2
<i>Arachis hypogaea</i>	Peanut	10.0	1.4	52
<i>Cicer arietinum</i>	Chick pea	9.6	1.8	19.0
<i>Cucurbita Pepo</i>	Squash	3.8	2.4	34.6

The distribution of vitamins within the seed has been thoroughly studied for a number of species. Table XI, taken from Rytz (1939),

TABLE XI
Thiamine Content of Pea Seeds during Development and at Maturity
After Rytz, 1939

Organ	State of maturity	Thiamine content, γ /g. dry weight
Whole seed.....	Immature	19
Whole seed.....	Mature	15
Cotyledons.....	Mature, not dry	24
Cotyledons.....	Mature, dry	11
Embryos.....	Mature dry seeds	16

shows that the thiamine content of the whole pea seed reaches a maximum at maturity and before drying out of the seed. In the mature dry seed, the cotyledons contain 11 γ /g. and the embryo 16 γ /g. dry weight.

TABLE XII
Distribution of Vitamins of the B Complex in Cereal Seeds

Species	Part	Concentration of vitamin, γ /g. dry weight			
		Thia- mine	Ribo- flavin	Nia- cin	Reference
<i>Avena sativa</i> (oat)	Endosperm	11.7	1.6	9.5	McVeigh, 1944
	Root of embryo	20.6	13.7	77.5	
	Coleoptile of embryo	12.5	20.0	107	
<i>Triticum vulgare</i> (wheat)	Endosperm	4.3	Pulkki and Puutula, 1941
	Embryo	13.9	
<i>Triticum vulgare</i> (wheat)	Scutellum	120	15	Hinton, 1942
	Embryo	12	15	
<i>Triticum vulgare</i> (wheat)	Endosperm	1.8	Ward, 1943
	Scutellum	148	
	Embryo	19.1	
<i>Secale cereale</i> (rye)	Whole grain	2.4	Hinton, 1944
	Scutellum	114	
	Embryo	6.9	

Table XII shows that thiamine is relatively low in concentration in the endosperm of cereal seeds, is higher in the embryo and is exceedingly high in concentration in the scutellum, concentrations of up to nearly 150 γ /g. being reported. Since the scutellum makes up approximately 1.5% of the dry weight of the seed, over one-half of the total thiamine of the seed is contained in this one tissue. Riboflavin and niacin are also present in higher concentration in the embryo than in the endosperm. The riboflavin of the endosperm is found in highest concentration in the aleurone layer according to Somers *et al.* (1945).

VII. TRANSLOCATION OF THE B VITAMINS

1. Vegetative Plants

That vitamins of the B complex are translocated in the plant can readily be shown by the use of the girdling technique, a standard physiological tool in the study of translocation of materials within the plant. The method of girdling which has been used primarily is one in which the living tissues of the stem or petiole are killed over a short region by a jet of steam. Translocation through the phloem is then blocked at this point although movement of water and salts through the nonliving elements of the xylem continues. Substances which are normally trans-

located through the phloem accumulate at the girdle and from the position of the accumulation the normal direction of transport of the material can be deduced. If the stems of tomato plants are girdled near the root level, thiamine accumulates rapidly in the region of stem immediately above the girdle and is gradually depleted not only from the stem below the girdle but from the entire root system of the plant (Bonner, 1942e). It would appear therefore that thiamine is normally transported from the aerial portions of the plant toward the root, a conclusion similar to that drawn by Burkholder and McVeigh (1942) from girdling experiments with woody plants. Thiamine transport in the vegetative plant occurs not only toward the root but also toward the apical growing point, as has been shown by the accumulation of thiamine below girdles made near the growing point of vegetative tomato plants (Bonner, 1942e). Since the only evidence available concerning the growth factor requirements of isolated buds (Loo, 1945) indicates that such tissue does not require added thiamine for growth *in vitro*, the physiological significance of this acropetal transport is not clear.

The synthesis of thiamine in the plant takes place in the leaves as has been shown in a variety of ways with the tomato (Bonner, 1942e). Thus the transport of thiamine through the stem toward the root occurs only in plants which possess leaves. If petioles are girdled, accumulation of thiamine occurs on the laminar side of the girdle. Thiamine formation and export of thiamine occurs most vigorously in the mature leaves whereas little or no transport occurs from young rapidly expanding leaves. These leaves must nevertheless be capable of thiamine synthesis, since they increase in total thiamine content in the absence of any detectable thiamine transport to them from the older leaves.

Pyridoxine, like thiamine, is produced in the leaves of tomato plants and is exported from the leaves to the roots (Bonner and Dorland, 1943b). Rapid accumulation of pyridoxine takes place in the stem above a basal girdle. Riboflavin, on the contrary, does not accumulate to any extent in the region of stem above a girdle and would hence appear not to be transported toward the roots (Bonner and Dorland, 1943a) while pantothenic acid accumulates only to a moderate extent. Neither riboflavin nor pantothenic acid is transported toward the apical growing point. Riboflavin is not exported from the leaves of tomato plants, and it appears that the various vegetative tissues of the tomato must all possess the ability to synthesize their own supply of this factor as has been shown above for the roots. Pantothenic acid is translocated from tomato leaves and the leaves are undoubtedly an active site of pantothenic acid synthesis. Translocation of pantothenic acid from the leaves to the roots occurs, however, only to a limited extent. In summary, of the four

vitamins whose translocation in the vegetative tomato plant has been studied, thiamine and pyridoxine, which the root is unable to synthesize and which are required as root growth substances, are formed in the leaves and vigorously transported to the roots. Riboflavin, which is synthesized by roots, appears to be synthesized by other tissues also and

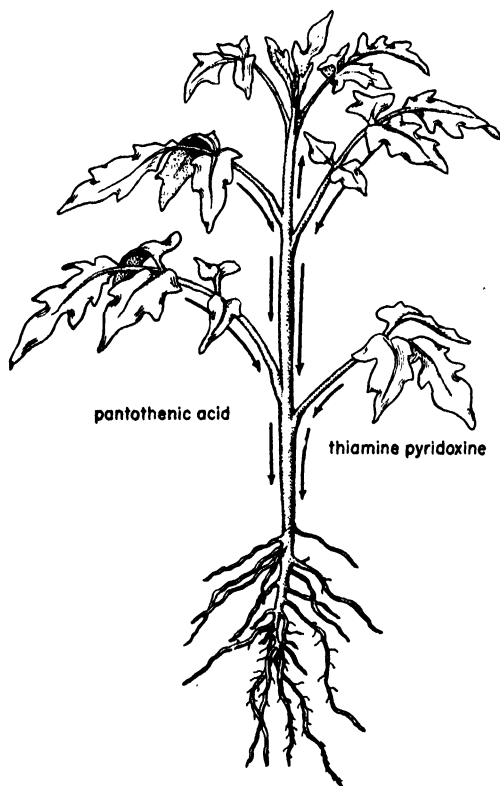


FIG. 1.—Diagrammatic representation of the directions of transport of thiamine, pyridoxine, and pantothenic acid in the tomato plant. Riboflavin is not translocated.

is not translocated appreciably, whereas pantothenic acid represents an intermediate case. This vitamin, which is not required as a root growth factor, is exported from the leaves but is translocated toward the roots only to a limited extent.

The synthesis and translocation of purines has been studied primarily with respect to the methylated purines, caffeine, and theobromine. These alkaloids as well as xanthine, hypoxanthine, guanine, and adenine occur primarily in leaves and the purines themselves are probably universal constituents of leaves. The methylated purines are distributed

in the plant as are the B vitamins, with highest concentrations being found in the youngest leaves and lower concentrations but greater total amounts in the older leaves (Weevers, 1930). In the stem the methylated purines are found only in the bark and the suspicion must be that the substances may be translocated in the phloem, although direct evidence, aside from the inconclusive work of Weevers (1930, 1932) is meager. Went (1932) found caffeine to be taken up by living cells of *Impatiens* stems and translocated polarly in the apical direction. Further studies on the role of purines as leaf growth factors should include investigations of the site of synthesis and the subsequent translocation of the substances.

The site of synthesis of other members of the B complex is as yet unknown. It may be suspected by analogy with the results already obtained that niacin may be formed in leaves, and then translocated to the roots, particularly in species whose roots are incapable of niacin synthesis. Biotin, *p*-aminobenzoic acid, the purines, and other factors which are synthesized in roots may, like riboflavin and pantothenic acid, be translocated from leaves to roots only sluggishly if at all.

2. Translocation of Vitamins into Reproductive Organs

It has been shown above that vitamins of the B complex including thiamine, pyridoxine, niacin, biotin, and pantothenic acid are essential factors in embryo and/or seedling development as judged by *in vitro* culture experiments. The vitamins supplied to the developing embryo must therefore derive either from tissues of the ovary other than the embryo or from the vegetative portions of the plant. Similarly the

TABLE XIII

Apparent Translocation of Thiamine from Glumes, Stems, and Leaves to the Fruit, during the Development of Wheat
After Geddes and Levine, 1942

Days after flowering	Dry weight per grain mg.	Thiamine content γ /g.			Thiamine content γ /plant			Total
		Fruits	Glumes etc.	Leaves and stem	Fruits	Glumes etc.	Leaves and stem	
7	3.4	7.2	3.3	3.2	0.9	1.1	4.5	6.5
10	6.5	7.6	2.8	3.1	1.9	1.0	4.2	7.0
14	12.7	6.6	1.7	2.7	3.4	0.6	3.4	7.3
17	15.3	5.9	1.5	2.4	3.6	0.5	2.9	6.9
21	19.2	5.7	1.4	2.1	4.3	0.4	2.2	7.0
24	20.7	5.2	1.1	1.8	3.9	0.3	1.8	6.0
28	23.0	5.2	0.6	1.2	4.2	0.2	1.1	5.5

vitamins stored in the cotyledons or endosperm and used by the seedling in seedling growth may derive either from synthesis *in situ* or from translocation from the leaves or other vegetative organs. The source of the thiamine stored in developing wheat grains has been considered by Geddes and Levine (1942). As the seeds increase in size and in total thiamine content, the thiamine of the glumes and rachis, stem, and leaves together decreased by an amount approximately equaling that appearing in the seeds, as is shown in Table XIII. It would seem that in wheat new synthesis of thiamine ceases at or shortly after the beginning of seed development and that filling of the seeds with thiamine is accomplished by translocation of the vitamin from the leaves and other vegetative tissues. In the pea, on the other hand, the synthesis in the vegetative plant continues during seed development so that withdrawal of thiamine toward the seed, if it occurs, is masked (Rytz, 1939).

The mechanisms by which B vitamins other than thiamine accumulate in the tissues of the fruit, seed or embryo, whether by translocation or by synthesis *in situ* have not been investigated. It would be of the greatest interest to study this aspect of the vitamin economy of the higher plant, particularly in view of the light which might then be thrown on the possible role of vitamins as controlling agents in fruit and seed development.

3. Behavior of B Vitamins in Seeds during Germination

Related to the question of the vitamin economy of the embryo during development is the status of the B vitamins during seed germination. In the germination of pea seeds from which the reserve of the cotyledons has been removed, thiamine is not synthesized to an appreciable degree when the plants are grown in the dark (Bonner and Greene, 1938). When the plants are grown in the light, however, major increases in thiamine occur, as shown in Table XIV. A similar requirement of light

TABLE XIV
Synthesis of Thiamine in Cotylectomized Pea Embryos Grown in Light or Dark
After Bonner and Greene, 1938

Condition	Plant part	Vitamin B ₁ content, γ /part. Days after removal of cotyledons and initiation of experiment		
		0	4	8
Dark.....	1-cm. Root tip	15	15	18
	Apical bud and 2 leaves	28	32	32
Light.....	1-cm. Root tip	15	16	46
	Apical bud and 2 leaves	28	36	100

for thiamine synthesis in the seedling also obtains for germinating tomato seeds (Bonner, 1941) and for wheat (Hoffer *et al.*, 1946). Even in seeds grown in the light the seedling plant may be totally dependent on its reserve of thiamine during the initial stage of germination, as shown by the data of Table XV for black-eyed peas and lima beans. In cotton, on the other hand, some synthesis of thiamine may occur in the light even within 36 hours. Riboflavin is synthesized in the germinating seeds

TABLE XV
Synthesis of Various Vitamins in Germinating Seedlings in the Light
After Cheldelin and Lane, 1943

Vitamin	Vitamin contents, γ /g. dry weight					
	Blackeyed peas		Lima beans		Cotton	
	0 time	36 hrs.	0 time	36 hrs.	0 time	36 hrs.
Thiamine.....	8.5	12	5.8	4.7	3.2	4.9 ¹
Riboflavin.....	1.5	3.9 ¹	1.4	2.4 ¹	2.3	2.8
Niacin.....	14	24 ¹	11	16 ¹	16	24 ¹
Pantothenic acid...	11	13	9	11	11	22 ¹
Pyridoxine.....	2.0	3.3 ¹	6.0	6.7	0.9	2.6 ¹
Biotin.....	0.22	0.31	0.11	0.16 ¹	0.29	0.28
Inositol.....	2600	4400 ¹	1800	3100 ¹	3400	2800
Folic acid.....	7.8	4.6	3.0	2.7	3.0	7.9 ¹

¹ Probable significant synthesis of vitamin during the experimental period. The data given do not permit of correction for the decreased dry weight of the seed during germination.

of wheat, corn, and beans even in the dark, according to Burkholder and McVeigh (1942), and Table XVI gives data on the behavior of several vitamins in dark-germinated seeds of soybean and mung bean. Similar synthesis of riboflavin occurs during germination in the light for black-eyed peas and lima beans (Cheldelin and Lane, 1943, Table XV). Niacin is synthesized in seedlings in the dark in the case of soy bean and mung bean (Table XVI) and during the initial stages of germination in the light in black-eyed peas, lima beans, and cotton (Table XV). Pantothenic acid, pyridoxine, biotin, inositol, and folic acid are synthesized during the early germination of some species, but not in others (Tables XV and XVI). In their study of the behavior of the B vitamins in seeds of soy and mung bean germinating in the dark, Burkholder and McVeigh (1945b) found no synthesis of folic acid but large increases in riboflavin, niacin, and inositol. Only small increases in pyridoxine were found while pantothenic acid and biotin behaved differently in different cases (Table XVI).

In summary it would seem that the seed, while it may be able to synthesize a portion of the B vitamins during germination, is nevertheless in general dependent on the stored reserve for one or more members of the complex. When germination occurs in the dark this is true of thiamine but not of riboflavin or in general of niacin although the niacin requirement of pea embryos implies a lack of synthetic ability for this vitamin in the particular instance of the pea embryo. The mobilization

TABLE XVI

*Behavior of B Vitamins in the Germination of Soy and Mung Beans in the Dark.
Germinated for 4 days
After Burkholder and McVeigh, 1945b*

Vitamin	Vitamin content, γ /g. dry weight			
	Soy bean (Peking)		Mung bean	
	Dormant	Germinated	Dormant	Germinated
Thiamine.....	16.2	18.8	8.2	8.4
Riboflavin.....	3.8	4.9 ¹	3.0	14.4 ¹
Pyridoxine.....	12.6	13.2	11.4	13.3
Niacin.....	21.8	31.5 ¹	23.1	66.5 ¹
Pantothenic acid.....	36.3	34.4	16.4	33.7 ¹
Inositol.....	2436	3784 ¹	1849	3649 ¹
Folic acid.....	1.86	0.71	2.47	1.96
Biotin.....	0.90	1.09	0.22	0.68 ¹

¹ Probable significant synthesis in total amount of vitamin per plant.

of thiamine from endosperm to embryo during germination has been demonstrated by McVeigh (1944) in *Avena sativa*. The embryo also mobilizes riboflavin and niacin, but in addition to the amounts of the two vitamins derived in this manner the tissues of the embryo appear to be able to synthesize them even in the dark. The most comprehensive study of vitamin mobilization in the germinating seedling is that of Hoffer, Alcock, and Geddes (1946) on the redistribution of these substances in wheat. Wheat seeds were allowed to germinate in the dark and the total amounts of thiamine in the endosperm and embryo were followed over an 18-day period. While total thiamine remained constant, indicating lack of new thiamine synthesis, the thiamine content of the embryo increased from an initial 11% of the total to 60% of the total after 18 days (Table XVII). This accumulation of thiamine in the embryo was attended by a corresponding loss of thiamine from the endosperm.

TABLE XVII

Translocation of Thiamine from Endosperm to Embryo during the Germination of Wheat Seeds in the Dark

After Hoffer, Alcock, and Geddes, 1946

Portion	Thiamine content γ /100 plants			
	Days incubation			
	2	4	9	18
Embryonic axis.....	1.4	5.8	6.5	9.6
Half of endosperm including scutellum.....	10.5	7.6	5.8	3.6
Brush half of endosperm.....	1.8	1.6	1.3	1.0
Total thiamine.....	13.7	14.4	13.6	14.4

VIII. B VITAMINS AS GROWTH FACTORS FOR INTACT PLANTS

Since certain of the B vitamins are required as growth factors by roots of several species and since, as shown above, certain of the vitamins, particularly thiamine, are synthesized only slowly in the germinating seed, it is natural to inquire whether any of the B vitamins may be limiting factors in the growth of the intact green plant. It is known that most if not all species of higher plants (with the exception of saprophytes and parasites) can be grown in the light in media containing only inorganic nutrient salts. It is clear therefore that at most the further addition of vitamins might be expected to promote growth; it would not be expected that vitamins would in any case be essential to the growth of the intact plant in the way in which thiamine, pyridoxine, and niacin are required in the nutrition of isolated roots. Extensive investigation of this question has revealed that available thiamine is not a limiting factor in the growth of most species and that thiamine can become a limiting factor only in a few particular species or under particular environmental circumstances.

Evidence that selected species may respond to additions of thiamine with increased growth was first presented by Bonner and Greene (1938). Thiamine at concentrations of 0.01 to 1 mg./l. water or nutrient was supplied to sand culture- or soil-grown plants, including *Camellia* and other relatively slow growing woody species, and increases in shoot and root growth of 30 to over 100% were reported. More recent data have also confirmed the effect of thiamine on the growth of *Camellia japonica* in nutrient culture in the greenhouse (Bonner, 1945). In subsequent papers (Bonner and Greene, 1939, Bonner, 1943b) additions of thiamine were reported to increase the growth of herbaceous species grown in

nutrient culture. Thus in a series of 15 experiments *Cosmos* plants were found to produce 27% more dry weight of tops when supplied thiamine than when thiamine was omitted from the nutrient. The growth promoting effect of thiamine was obtained, however, only under conditions of relatively low temperature such as a continuous temperature of 68°F. When the plants were grown under higher temperatures, growth was greatly increased and no response to thiamine was evident. That the growth promoting effect of thiamine on *Cosmos* is in general reproducible is shown by the results of 28 experiments with *Cosmos* carried on in a cooperative survey by 15 different investigators scattered widely over the United States (Cooperative Report, 1941). Twenty-two of these experiments yielded growth increases as a result of thiamine addition, the mean increase in top dry weight amounting to 27%. The dependence of response to thiamine on environmental conditions (Bonner, 1943b) no doubt accounts for the failure of certain investigators to obtain any effects with application of thiamine to *Cosmos* (Hamner, 1940; Arnon, 1940, and others). Positive effects of thiamine have been reported for other species, as summarized in Table XVIII. Thiamine has also proved to act as a growth promoting factor for green algae in sterile culture (Van Overbeek, 1940, Ondratschek, 1940a-d, 1941a, b). In general, however, application of thiamine to cultivated species is without any apparent effect on growth. Thus, in the cooperative experiment referred to above, only two of sixteen species showed any growth response to thiamine application. Negative results of thiamine application have been reported for orange trees (Parker, Turrell, and Bonner, 1941), tomato and pea (Bonner and Greene, 1939) and for other species, of which a general summary is given in Table XIX.

It must be concluded that while thiamine may be a limiting factor in a few scattered species or under particular conditions, still availability of this substance does not in general limit plant growth. This emphasizes the hormonal nature of thiamine in the plant. Even though the thiamine supply of the tomato root, for example, is dependent on the transport from the leaves, still the leaves are apparently able to provide the roots with an ample supply of thiamine and the plant taken as a whole is thiamine autotrophic. The thiamine supply of the roots may in some cases even be so abundant as to permit of extensive loss of thiamine to the medium in which the plant is grown, as shown by West (1939) in the case of flax seedlings, the roots of which appear to excrete thiamine as well as biotin.

The effects of vitamins other than thiamine on the development of intact green plants have been but little investigated. Niacin and pyridoxine have been reported to influence growth of *Cosmos* (Bonner

and Bonner, 1940) under the same conditions of unfavorably low temperature which permit of response to thiamine. Adenine was also found to be effective in promoting growth and dry weight accumulation of *Cosmos*, leaf size in particular being affected. Niacin is ineffective in increasing

TABLE XVIII

Summary of a Portion of Experiments Showing a Growth-Promoting Effect of Thiamine Application on Growth of Various Species of Plants

Species	Common name	Reference
<i>Agrostis tenuis</i>	Bent grass	Bonner and Greene, 1939
<i>Aleurites Fordii</i>	Tung	Bonner and Greene, 1938
<i>Allium Ceba</i>	Onion	Borgström, 1939
<i>Allium fistulosum</i>	Spring onion	Borgström, 1939
<i>Anethum graveolens</i>	Dill	Murneck, 1941
<i>Arbutus Unedo</i>	Strawberry tree	Bonner and Greene, 1938
<i>Atropa Belladonna</i>	Belladonna	Zopf, 1940
<i>Begonia</i> sp.....	Begonia	Fraps and Fudge, 1942
<i>Beta vulgaris</i>	Sugar beet	William, 1939
<i>Bougainvillea glabra</i>	Bougainvillea	Bonner and Greene, 1938
<i>Brassica alba</i>	White mustard	Bonner and Greene, 1939
<i>Brassica nigra</i>	Black mustard	Bonner and Greene, 1939
<i>Bryophyllum degremontianum</i>	Bonner and Greene, 1938
<i>Callistephus chinensis</i> (flat grown).....	China aster	Hitchcock and Zimmerman, 1941
<i>Camellia japonica</i>	Camellia	Bonner and Greene, 1938
<i>Capsicum</i> sp.....	Ornamental pepper	Bonner, 1945
<i>Cattleya Harrisoniae</i>	Murneck, 1941
<i>Cattleya hybrid</i>	Meyer, 1944
<i>Cedrus libani</i>	Cedar of Lebanon	Bonner and Greene, 1939
<i>Ceratonia Siliqua</i>	Carob	Bonner and Greene, 1939
<i>Correa ventricosa</i>	Bonner and Greene, 1939
<i>Cosmos sulphureus</i>	Cosmos	Bonner and Greene, 1939
<i>Daphne odorata</i>	Murneck, 1941
<i>Eucalyptus ficifolia</i>	Scarlet-flowering gum	Bonner, 1943b
<i>Lathyrus odoratus</i>	Sweet pea	Bonner and Greene, 1939
<i>Myrsine africana</i>	Bonner and Greene, 1939
<i>Poa pratensis</i>	Kentucky blue-grass	Cooperative report, 1941
<i>Poa trivialis</i>	Rough blue-grass	Cooperative report, 1941
<i>Prunus ilicifolia</i>	Catalina cherry	Bonner and Greene, 1939
<i>Ricinus communis</i>	Castor-bean	Bonner and Greene, 1939
<i>Rodriguezia</i> sp.....	Zopf, 1940
<i>Valeriana officinalis</i>	Valerian	Meyer, 1944
<i>Viola tricolor</i>	Pansy	Neipp, 1943
<i>Xanthium pennsylvanicum</i>	Cocklebur	Fraps and Fudge, 1942
		Bonner and Greene, 1939

TABLE XIX

Summary of a Portion of Experiments Showing no Effect of Thiamine Application on Growth of Various Species of Plants

Species	Common name	Reference
<i>Agrostis tenuis</i>	Colonial bentgrass	Clark, 1942
<i>Antirrhinum majus</i>	Snapdragon	Ahlgren, 1941
<i>Aster</i> sp.....	Aster	Fraps and Fudge, 1942
<i>Aster</i> sp.....	Aster	Fraps and Fudge, 1942
<i>Avena sativa</i>	Oats	Laurie and Kiplinger, 1941
<i>Begonia</i> sp.....	Begonia	Lundegårdh and Wikén, 1943
<i>Beta vulgaris</i>	Sugar beet	Templeman and Pollard, 1941
<i>Beta vulgaris</i>	Beet	Fraps and Fudge, 1942
<i>Brassica alba</i>	White mustard	Lundegårdh and Wikén, 1943
		Bouillenne and Roubaix, 1942
		Minnum, 1941a
		Clark, 1942
		Arnon, 1940
		Hamner, 1940
<i>Brassica Napobrassica</i>	Rutabaga	Minnum, 1941a
<i>Brassica oleracea</i>	Cabbage	Hamner, 1940
<i>Brassica oleracea</i>	Cauliflower	Minnum, 1941a, b
<i>Callistephus chinensis</i>	China aster	Hitchcock and Zimmerman, 1941
<i>Chilopsis linearis</i>	Desert-willow	Donnelly, 1941
<i>Chrysanthemum morifolium</i>	Chrysanthemum	Swartz, 1941
		Fraps and Fudge, 1942
		Laurie and Kiplinger, 1941
		Cooperative experiment, 1941
<i>Cineraria cruenta</i>	Cineraria	Fraps and Fudge, 1942
<i>Citrus sinensis</i>	Orange	Parker <i>et al.</i> , 1941
<i>Cucurbita Pepo</i>	Squash	Minnum, 1941a
<i>Dahlia pinnata</i>	Dahlia	Fraps and Fudge, 1942
		Hamner, 1940
<i>Dianthus Caryophyllus</i>	Carnation	Cooperative experiment, 1941
<i>Frazinus velulina</i>		Donnelly, 1941
<i>Gardenia jasminoides</i>	Gardenia	Fraps and Fudge, 1942
		Laurie and Kiplinger, 1941
<i>Gladiolus</i> sp.....	Gladiolus	Reeves, 1942
<i>Gleditsia triacanthos</i>	Sweet locust	Donnelly, 1941
<i>Gossypium hirsutum</i>	Cotton	Woodhouse and Morris, 1942
<i>Helianthus</i> sp.....		Gisiger, 1943
<i>Helianthus</i> sp.....		Fraps and Fudge, 1942
<i>Holcus Sorghum</i>	Kafir	Myers <i>et al.</i> , 1941
<i>Hordeum vulgare</i>	Barley	Lundegårdh and Wikén, 1943
<i>Juglans Hindsii</i>		Donnelly, 1941
<i>Lactuca sativa</i>	Lettuce	Haber and Edgecombe, 1940
		Arnon, 1940

TABLE XIX.—(Continued)

Species	Common name	Reference
<i>Lemna</i> sp.....	Duckweed	Gorham, 1941
<i>Linum usitatissimum</i>	Flax	Gisiger, 1943
<i>Lobularia maritima</i>	Sweet alyssum	Cooperative experiment, 1941
<i>Lolium perenne</i>	Perennial rye-grass	Ahlgren, 1942
<i>Lycopersicon esculentum</i>	Tomato	Bonner and Greene, 1939 Templeman and Pollard, 1941 Arnon, 1940 Minnum, 1941a
<i>Malus domestica</i>	Apple	Tukey and Brase, 1941
<i>Olea verrucosa</i>	Donnelly, 1941
<i>Oryza sativa</i>	Rice	Minarik, 1942
<i>Phaseolus vulgaris</i>	Bean	Minnum, 1941a Gisiger, 1943
<i>Phleum pratense</i>	Timothy	Ahlgren, 1942
<i>Pinus ponderosa</i>	Yellow pine	Fowells, 1943
<i>Pisum sativum</i>	Pea	Bonner and Greene, 1939
<i>Poa pratensis</i>	Kentucky blue-grass	Ahlgren, 1941, 1942 Laurie and Kiplinger, 1941
<i>Poa trivialis</i>	Rough blue-grass	Ahlgren, 1941
<i>Prosopis juliflora</i>	Mesquite	Donnelly, 1941
<i>Prunus Persica</i>	Peach	Tukey and Brase, 1941
<i>Pyrus betulaefolia</i>	Pear	Tukey and Brase, 1941
<i>Raphanus sativus</i>	Radish	Minnum, 1941a, b Swartz, 1941 Hamner, 1940
<i>Rosa multiflora</i>	Rose	Tukey and Brase, 1941
<i>Rosa</i> sp.....	Fraps and Fudge, 1942
<i>Rosa</i> sp.....	Laurie and Kiplinger, 1941
<i>Secale cereale</i>	Rye	Lundegårdh and Wikén, 1943
<i>Simmondsia californica</i>	Goat-nut	Donnelly, 1941
<i>Spirodela polyrrhiza</i>	Duckweed	Gorham, 1945
<i>Tagetes lucida</i>	Marigold	Swartz, 1941
<i>Trifolium repens</i>	White clover	Ahlgren, 1942
<i>Triticum aestivum</i>	Wheat	Gisiger, 1943
<i>Vaccinium corymbosum</i>	Blueberry	Kramer and Schrader, 1942
<i>Vinca</i> sp.....	Periwinkle	Cooperative experiment, 1941
<i>Xanthium pennsylvanicum</i>	Cocklebur	Arnon, 1940
<i>Xanthium pennsylvanicum</i>	Cocklebur	Hamner, 1940
<i>Zea Mays</i>	Corn	Gisiger, 1943 Woodhouse and Morris, 1942 Minnum, 1941a Myers <i>et al.</i> , 1941
<i>Zinnia elegans</i>	Fraps and Fudge, 1942 Hamner, 1940

growth of tomato (Templeman and Pollard, 1941). Riboflavin has been suggested to increase growth of pot cultures of egg plant and tobacco (Dennison, 1940) but this effect has not been obtained by other investigators (Bonner, 1940c, Minnum, 1941b). Pyridoxine and pantothenic acid are ineffective in causing growth increases with radish or cauliflower (Minnum, 1941b).

A special case of vitamin requirements by intact green plants is presented by the orchids. In this group, the seeds are ordinarily germinated on nutrient media in aseptic culture and the young plants transferred to purely mineral substrate only after the attainment of a considerable size. With many forms, sugar and inorganic salts provide the essential factors for seed germination and seedling development. In other cases, however, the addition of further factors is essential to development, as has been shown by Burgeff (1934) for orchids of the *Vanda* group and by Schaffstein for *Phalaenopsis* (1938). Identification of the active factors has been made in the case of a *Cattleya* hybrid which failed to germinate on medium containing only purified sugar and salts. Addition of pyridoxine permitted good seed germination but only poor further growth, whereas addition of niacin permitted both good germination and excellent development of the seedlings (Noggle and Wynd, 1943). Thiamine has been found to increase the rate of development of seedlings of *Rodriguezia* and *Cattleya* species (Meyer, 1944).

IX. VITAMINS OF THE B COMPLEX AS GROWTH FACTORS FOR CUTTINGS

The fact that thiamine, niacin, and pyridoxine are required as growth factors for isolated roots immediately suggests the possibility that these same substances might be of utility in promoting the growth of roots in the rooting of cuttings. It is known that the application of indoleacetic acid or other compounds of the auxin group to the basal portion of cuttings results in the initiation of root primordia in many species. The auxins are, however, inhibitory to the further development of the root, and it appears to be an essential feature of root induction by auxins that the active substance must have long disappeared before actual outgrowth of the new roots can take place (Cooper, 1938). In particular instances, auxin application to the cutting results in the formation of root primordia which fail to grow out into roots. Such a situation has been described by Went *et al.* (1938) in the *Camellia*. In a particular experiment cuttings treated with indoleacetic acid which had formed root primordia but on which no roots had grown out were retreated with thiamine (1 mg./l.). Within 24 hours the primordia present made visible growth and ultimately grew out into normal roots. Similar experiments with *Camellia*,

Dracena, laurel, lemon, *Chamaelaucium*, and other species have been reported by Warner and Went (1939). It would appear that with the particular species and individuals used, the thiamine in the cutting and available at the region of root initiation may have been insufficient to provide for the elongation of new roots. It has been stressed by Warner and Went (1939) that the promotion of root elongation by thiamine in auxin-treated cuttings appears to apply only to particular species and conditions of the tissue, and this view has been substantiated by other workers. Thus Deuber (1940) found a considerable increase of root growth in hemlock cuttings after thiamine application while no effect was evident with Norway spruce. *Fraxinus* cuttings have given a small but measurable response to thiamine (Thimann and Delisle, 1939). Small or negative effects have been obtained with thiamine treatment of cuttings by Haber and Edgecombe (1940) and Tincker and Unwin (1939). Pearse (1939) has reported increased root growth as a result of thiamine application to auxin-treated cuttings of pear, quince, apple, plum, and cherry, while Hitchcock and Zimmerman (1940) have reported that thiamine in combination with auxin increases the rooting of some species.

Pyridoxine, like thiamine, promotes the rooting of the cuttings of individual species as reported by Stoutemeyer (1940) who found nine species to respond to this vitamin. Cuttings of *Lonicera nitida* in particular rooted in response to pyridoxine additions even without preliminary application of auxin. Rooting in this species would seem to be limited primarily by available pyridoxine.

The application of biotin to stems treated with indoleacetic acid has been found to increase the number of roots formed by cuttings of etiolated pea seedlings (Went and Thimann, 1937). The effect of biotin differs from that of thiamine, which is effective only in promoting outgrowth of roots, in that biotin actually promotes the formation of root primordia. Inositol which was also tested was ineffective.

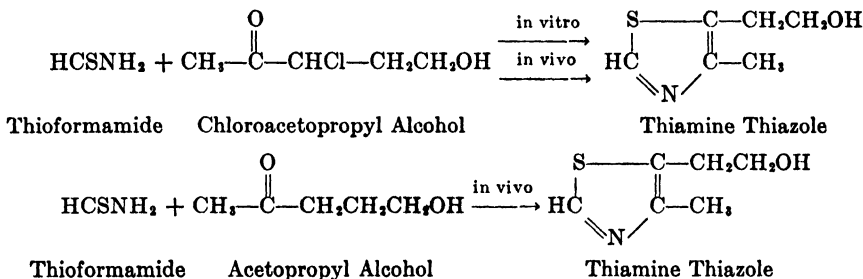
This summary shows that in particular instances the root growth substances thiamine or pyridoxine may be limiting factors in the process of root growth in cuttings. No detailed investigation as yet has attempted to correlate these effects with vitamin content or status of the cutting. Other members of the B complex and in particular niacin also remain to be investigated as factors in the rooting of cuttings.

X. BIOCHEMISTRY AND METABOLISM OF THE B VITAMINS

1. Thiamine

It has been shown above that the synthesis of thiamine may take place in roots at the expense of the free thiazole and pyrimidine moieties

and the same is true of certain microorganisms as *Phycomyces Blakesleeanus* (Bonner and Buchman, 1939b). The biosynthesis of thiamine is then resolved into the course of biosynthesis of the thiazole and pyrimidine portions of the molecule. A possible course of the biosynthesis of the thiazole portion of the thiamine molecule in the plant is suggested by experiments of Bonner and Buchman (1938) with isolated pea roots. The thiamine thiazole may be synthesized in the test tube from thioformamide and chloroacetopropyl alcohol. Isolated pea roots are able



to utilize a mixture of these two substances to satisfy their thiazole requirement and in fact synthesize the vitamin thiazole from the mixture. Acetopropyl alcohol, unlike chloroacetopropyl, does not react spontaneously with thioformamide to form the vitamin thiazole. A mixture of thioformamide and acetopropyl alcohol can, however, also be used by pea roots to satisfy their thiazole requirement, and here again actual synthesis of thiazole appears to take place in the presence of the two components. Whether this reaction is a general reaction by which thiazole is synthesized in plants is as yet unknown. *Phycomyces* is not able to carry out the condensation while *Neurospora* can carry it out only to a limited extent (Tatum and Bell, 1946). No evidence as to the mode of biosynthesis of the pyrimidine portion is available.

Thiamine pyrophosphate is the coenzyme of the enzyme pyruvate carboxylase, as was first shown by Lohman and Schuster in the case of yeast (1937). The enzyme carboxylase occurs in some tissues of higher plants including pea roots (Horowitz and Heegaard, 1941), seeds and seedlings of cereals (Bunting and James, 1941, Bartlett, 1942), tomato seedlings (Bartlett, 1942), cucurbit seeds and fruits (Vennesland and Felsher, 1946) and various leaves (Vennesland and Felsher, 1946). In the case of the pea root the enzyme contains cocarboxylase (Horowitz and Heegaard, 1941), and cocarboxylase has also been reported from a number of other species (Tauber, 1937). We may thus picture the overall course of thiamine metabolism in the higher plants in a preliminary way as follows:



Although the biochemical basis of thiamine action in the plant may in particular instances rest in part on the participation of cocarboxylase in pyruvate decarboxylation, still this system cannot be the only one in which thiamine is involved, since many tissues of higher plants are free of pyruvate carboxylase including spinach leaves (Bonner and Wildman, 1946) and various seeds, leaves, and fruits (Vennesland and Felsher, 1946). Oxalosuccinate carboxylase has been studied by Ochoa and Weisz-Tabori (1945) in animal tissue and found to contain cocarboxylase as a component, as does the system for oxidative decarboxylation of pyruvate by bacteria (Lipmann, 1937, 1939, 1941). In summary, the biochemical basis of thiamine activity in the plant undoubtedly resides in participation of cocarboxylase in one or more decarboxylating enzyme systems of the respiratory mechanism including possibly pyruvate carboxylase, α -ketoglutarate carboxylase, and perhaps others such as the oxalacetate carboxylase (Vennesland and Felsher, 1946).

The destruction of thiamine proceeds rapidly in cultures of *Phycomyces* by a reaction in which the thiazole portion of the molecule is inactivated with the liberation of an equivalent amount of free pyrimidine (Bonner and Buchman, 1939b).



The inactivation of the thiazole portion presumably proceeds by a reaction involving position 2 of the thiazole nucleus, since if the H of this position is substituted by CH_3 or NH_2 the resultant thiazole is not destroyed. It is probable that thiamine inactivation proceeds along similar lines in leaves since free uncombined pyrimidine has been found in leaves of all species investigated (Bonner, 1942b).

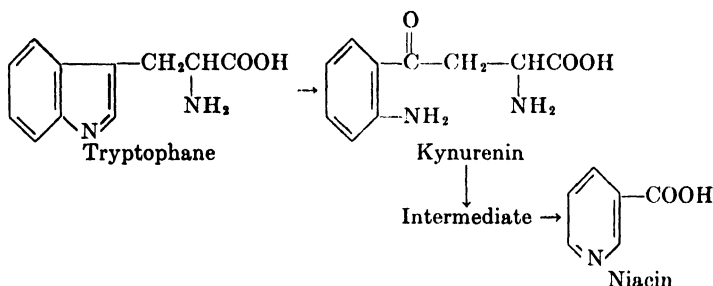
2. Riboflavin

Riboflavin functions as a component of nucleotides which are the prosthetic groups of the flavoprotein enzymes. Of the numerous flavoproteins which have been described, only one, diaphorase, which is capable of reoxidizing reduced cozymase, has been found thus far in higher plants. Lockhart (1939) has shown the presence of this enzyme in pea and bean seedlings and in potato tubers, while Okunuki (1940) has found the enzyme in pollen. Thus riboflavin appears to be in the plant as in other organisms a constituent of a respiratory enzyme, and in the particular case known, a constituent of an enzyme which acts as

an intermediary in the transport of electrons from codehydrogenases to cytochrome or other terminal oxidase systems. It is very probable that further work will reveal the presence of other flavoproteins in higher plant tissues.

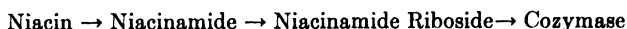
3. Niacin

The biosynthesis of niacin has been studied primarily in microorganisms rather than in higher plants, and on the basis of this work as well as from investigations on niacin synthesis in the rat, it is known that the synthesis of this vitamin proceeds from tryptophane with kynurenin as an intermediate (Beadle *et al.*, 1947; Rosen *et al.*, 1946).



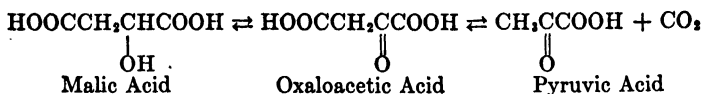
On the basis of the number of genetic blocks in mutant *Neurospora* strains for the synthesis of niacin from tryptophane it is clear that at least three steps are involved, one being before kynurenin and two being after kynurenin. Roots, which need an external supply of niacin, are unable to utilize tryptophane to replace the former.

Niacin is utilized in higher plants as in other organisms as a constituent of the nucleotide cozymase and possibly also of triphosphopyridine nucleotide. On the basis of the comparative growth factor requirements of microorganisms (Lwoff and Lwoff, 1937a, b) it is probable that the course of synthesis of cozymase from niacin proceeds through niacinamide, niacinamide riboside and finally to the coenzyme:



Enzymes of higher plant tissues for which cozymase is a coenzyme include malic dehydrogenase, alcohol dehydrogenase, and glutamic dehydrogenase, while a variety of other dehydrogenases must be suspected of occurring in the plant. Isocitric dehydrogenase of higher plants appears to require triphosphopyridine dinucleotide as a coenzyme (Berger and Avery, 1944).

The parsley root oxaloacetate carboxylase system of Gollub and Vennesland (1947) appears able to carry out the reversible reactions:

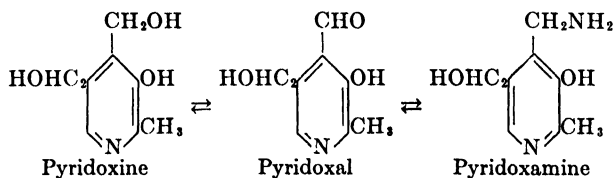


This reaction involves the triphosphopyridine nucleotide, probably in the oxaloacetate-malate step. An interesting if little understood relationship between niacin and indoleacetic acid has recently been reported by Galston (1947). Indoleacetic acid is inhibitory to the growth of isolated asparagus roots grown in culture in the dark while niacin is without effect. Niacin in the presence of indoleacetic acid greatly enhances the inhibitory effect of the latter.

In summary, niacin functions in the plant as a constituent of cozymase and triphosphopyridine nucleotide and as such is a constituent of essential respiratory enzymes.

4. Pyridoxine

It has been found by Snell *et al.* (1942) that pyridoxine is only one of a group of similar compounds and that the different forms possess widely differing activities as growth factors for various microorganisms. Thus pyridoxal, in which the 4 position is substituted by an aldehyde group, is highly active as a growth factor for *Lactobacillus casei*. With *Streptococcus lactis* and *S. faecalis*, both pyridoxal and pyridoxamine, in which the 4 position contains a methylamino group, are active as growth factors. With *Saccharomyces Carlsbergensis* and various fungi, pyridoxal, pyridoxamine, and pyridoxine are equally active. These facts suggest that pyridoxal may be the central compound and may be converted to pyridoxamine or pyridoxine. Organisms such as *S. lactis* would then be those able to carry out interconversion of pyridoxal and pyridoxamine while those as *L. casei* would be those unable to carry out any of the interconversions.



It has further been shown by Gunsalus *et al.* (1944a; 1944b) that pyridoxal can be phosphorylated by adenosine triphosphate in the presence of dead cells of *S. faecalis*. The resulting pyridoxal phosphate is the coenzyme of the amino acid decarboxylase of microorganisms, an enzyme which decarboxylates tyrosine, glutamic acid, arginine, glycine, ornithine, and other acids to the corresponding amines (Gunsalus and Bellamy, 1944b); Umbreit and Gunsalus, 1945; Gale and Epps, 1944).

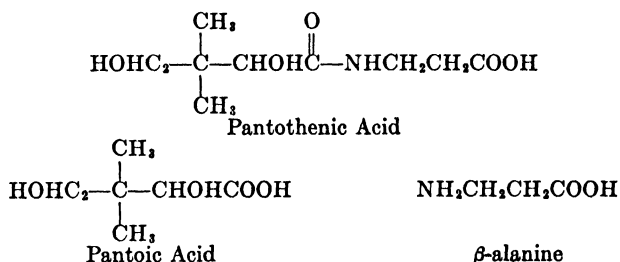
The glutamic acid decarboxylase of higher plants (Okunuki, 1937) has been shown by Schales *et al.* (1946) to have pyridoxal phosphate as a coenzyme.

Pyridoxal phosphate is also a coenzyme for the enzyme transaminase as has been shown by Lichstein *et al.* (1945) with *S. faecalis*. Transaminase has been shown to occur in higher plant tissues by Braunstein and Kritsman (1937), Virtanen and Laine (1941), Albaum and Cohen (1943), Rautanen (1946) and others. It is possible that with higher plant transaminase as with that of microorganisms, pyridoxal phosphate may act as a coenzyme.

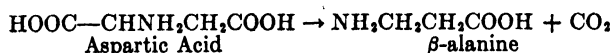
Pyridoxine is, then, active in the plant through its conversion to pyridoxal phosphate, a coenzyme of one and possibly two reactions important in N metabolism. In its possible role as a coenzyme in transamination, in particular, it may occupy a key position in the synthesis of amino acids and hence ultimately in the synthesis of the proteins of the plant.

5. Pantothenic Acid

Pantothenic acid, like thiamine, is made up of two moieties whose biosynthesis must be considered separately.



The β -alanine moiety of pantothenic acid is alone sufficient to satisfy the pantothenic acid requirement of many yeasts (Williams and Rohrmann, 1936) which are able to synthesize the pantoic acid fragment. Beta-alanine is doubtless produced in the plant by decarboxylation of aspartic acid, an enzyme for this reaction having been found in *Rhizobium* by Virtanen and Laine (1937).

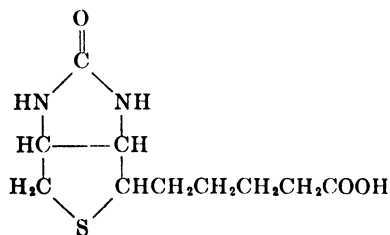


The pantoic acid moiety suffices to fulfill the pantothenic acid requirement of *Acetobacter*, which is able to synthesize the β -alanine portion (Underkofler *et al.*, 1943). Other organisms require both moieties (Tatum and Beadle, 1942) or intact pantothenic acid (Cheldelin *et al.* 1945). The reactions leading to pantoic acid synthesis are unknown.

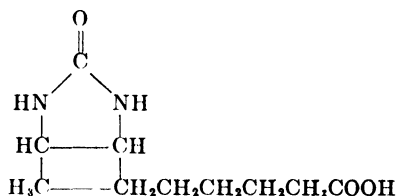
A biochemical role of pantothenic acid has been indicated by Lipmann *et al.* (1947) who has shown that this substance is a component of coenzyme A, the coenzyme of an enzyme which carries out acetylation. The role of coenzyme A appears to involve a reaction between acetate and ATP with the production of a compound having the properties of acetyl phosphate. Coenzyme A is also synthesized from pantothenic acid by the microorganism *Proteus morganii*, where it appears to function in the oxidation of pyruvate, presumably in utilization of acetate produced in oxidative decarboxylation of pyruvate (Novelli and Lipmann, 1947).

6. Biotin

That the course of biotin synthesis in nature is through desthiobiotin, a substance capable of replacing biotin as a growth factor for many yeasts and fungi has been shown by Tatum (1945) in experiments with two mutant strains of *Penicillium chrysogenum*. The growth factor requirement of one form was satisfied by either biotin or desthiobiotin while the second form responded to biotin alone. The second strain in addition produced a substance apparently identical with desthiobiotin which could serve as a growth factor for the first strain. Thus the strain which can utilize biotin alone is one which has lost the ability to transform desthiobiotin to biotin.



Biotin



Desthiobiotin

The addition of pimelic acid to cultures of *Phycomyces* (Bonner and Buchman, 1939a) or of other microorganisms (du Vigneaud *et al.*, 1942) greatly increases the production of biotin by these biotin autotrophic organisms. Further increases in biotin production in the case of *Phycomyces* were elicited by the addition of both pimelic acid and cysteine. Tatum (1945) in his work with *Penicillium* found that the production of desthiobiotin was increased twenty-fold by addition of pimelic acid. It is possible, therefore, that pimelic acid may be a precursor of desthiobiotin and that synthesis of biotin takes place along the following lines:



At present, however, nothing is known concerning the activity of these compounds in replacing biotin as a growth factor for higher plant tissues, as for example in embryo culture work. Lichstein and Umbreit (1947a, b) have shown that additions of biotin reactivate oxaloacetate carboxylase preparations of *Escherichia coli* which have been deactivated by maintaining the cells at pH 4 for 15–60 minutes at 37°C. A deaminase of the same organism, active on aspartate, serine, and other amino acids, may be similarly deactivated and subsequently reactivated by additions of biotin. The exact role of biotin in these enzymes as well as the possibility of biotin participation in still other enzyme systems remain to be elucidated.

7. Adenine and Other Purines

Older concepts of purine synthesis *in vivo* have been based on the reaction of urea with methyl glyoxal (Onslow, 1931), followed by reduction and dehydration to yield an intermediate perhaps isomeric with xanthine. Recent work shows, however, that purine synthesis, in the animal at least, is more complicated. In the formation of uric acid in the pigeon, isotopic carbon contained in CO₂, acetic acid, or glycine was incorporated in different positions in the purine nucleus (Sonne *et al.*, 1946). No evidence that urea is involved as a precursor could be obtained. It will be of interest to apply isotopic methods to the study of purine synthesis in the plant.

In the animal body adenine is oxidatively deaminated by the enzyme adenase to form hypoxanthine. Hypoxanthine is in turn attacked by the flavoprotein xanthine oxidase (which in turn contains adenine as a constituent) to yield xanthine which finally is oxidized to uric acid. Guanine similarly is oxidatively deaminated to xanthine by the enzyme guanase. These enzymes with the exception of guanase (Schittenhelm, 1909) have not been studied in higher plants and it is not known whether purine metabolism in the leaf or other plant tissue parallels that found in animal tissue. On the other hand, the fact that uric acid as well as allantoin and allantoic acid are found in plant tissues (Fosse *et al.*, 1926, 1932) suggests that purine breakdown in the plant may follow lines generally similar to those found in the animal.

The purine adenine which, as described above, is active as a leaf growth factor in pea and radish is a constituent of several varied biochemical systems in the plant. Thus adenine is a constituent of the nucleic acids of the cytoplasm and nucleus, of cozymase, of triphosphopyridine nucleotide, of the flavoproteins, and of adenosine di- and triphosphate. In the last role adenine is involved in plant metabolism, as

well as in microorganisms and in higher animal tissue, in transphosphorylation reactions such as phosphorylation of hexose (Bonner and Wildman, 1946).

XI. SUMMARY

The green plant taken as a whole is in general able to synthesize amounts of the B vitamins adequate for its own needs and is with few exceptions independent of any external source of these chemical substances. The individual parts of the plant, however, differ widely in their ability to synthesize the several vitamins of the B complex. Since present evidence tends to indicate that these substances are required by all living tissues, this means that a tissue lacking the ability to synthesize a given vitamin must obtain its supply from other tissues of the plant. Thus roots in general lack the ability to synthesize thiamine. Thiamine is required by roots and the thiamine which they need is produced in the leaves and translocated to the root system. The amounts of thiamine concerned are small, concentrations of the order of 5–20 γ /g. of dry tissue being the usual order in the vegetative plant. Thiamine therefore fulfills the requirements demanded of a hormone in that it is synthesized in one organ and transported to another where in small amounts it brings about a physiological response, e.g., growth. The same is known to be true of pyridoxine, likewise a growth factor for roots of many species. In other species pyridoxine may not function as a root growth hormone, since in the pea, for example, the root does not require external supplies of pyridoxine and is presumably able to synthesize the substance. Niacin is probably also to be regarded as a root growth hormone for species in which the roots are unable to synthesize the compound.

To state that thiamine, pyridoxine, or niacin function as root growth hormones does not imply that the same chemical substances may not function as correlative agents in quite different aspects of growth. The evidence available indicates that the growing embryo and its associated tissues such as the endosperm probably also lack the ability to synthesize thiamine but derive this substance by translocation from the leaves. Thiamine is required by the germinating seed and seedling plant, presumably also by the developing embryo. Thus it is entirely likely that thiamine may function not only as a root growth hormone but also as a hormone of seed and embryo development. Neither in the case of the root nor in the case of the seed and embryo is there evidence that the development on the intact plant of the organ in question is directly limited by available thiamine. On the contrary, in most species of plants root growth is controlled primarily by factors other than thiamine. Thiamine, while it is a hormone in the strict sense, is nevertheless not the

TABLE XX
A Summary of the Relationships of Vitamins of the B Complex in a Number of Phases of Plant Growth Development

Vitamin	Physiological behavior					
	Root-growth factor	Leaf-growth factor	Site of synthesis	Translocation in stem	Effect on embryo growth	Synthesis in germinating seed in dark
Thiamine.....	Required by most roots	Inactive	Leaf	Translocated toward roots, growing points, and seeds	Effective on pea	Not synthesized
Pyridoxine.....	Required by some roots	Inactive	Leaf, root in some cases	Translocated toward roots	Ineffective	Probably not synthesized
Niacin.....	Required by some roots	Inactive	Leaf? Root in some cases	Effective on pea	Synthesized
Pantothenic acid.....	Not required	Inactive	Leaf. Root?	Translocated sluggishly	Effective on pea, <i>Datura</i>	Synthesized in some cases, not in others
Riboflavin.....	Not required	Inactive	Leaf. Stem? Root	Not translocated	Ineffective	Synthesized
Biotin.....	Not required	Inactive	Leaf? Root	Effective on pea	Synthesized in some cases, not in others
Inositol.....	Not required	Inactive	Ineffective	Synthesized
Adenine and hypoxanthine	Not required	Active on radish pea	Leaf	Ineffective except on leaf growth
Folic acid.....	Not required	Leaf?	Not synthesized
						Required by rose callus in culture
						Required by callus of <i>Crotalaria</i>
						Cofactor in root initiation in pea

key or limiting factor in any developmental reaction of the intact plant which has as yet been studied.

The scattered bits of information brought together in this review indicate that pantothenic acid may, like thiamine, play a hormonal role in seed or embryo development, and the same may possibly be true of biotin. Critical evidence on the translocation of these materials in the plant is needed before a final conclusion can be reached.

It is of interest that in the factors thus far recognized as of importance in regulating growth of the mesophyll of leaves there is no overlapping with factors known to function as growth hormones of root or seed. Concerning the purines adenine and hypoxanthine, it is clear that they are not only active in promoting leaf growth but are also found in organs known to manufacture or store the native leaf growth factors. Critical evidence that the purines are actually the factors translocated in the plant is needed before a hormonal role can conscientiously be ascribed to these substances.

Riboflavin appears to be synthesized in all of the vegetative tissues of the plant and does not, so far as can be ascertained, possess any hormonal role in vegetative growth. Further, it is among the vitamins regularly synthesized in the germinating seed in considerable amounts and consideration must be given to the possibility that it may even be synthesized in the developing seed and embryo. The small amount of evidence available suggests that inositol may constitute a similar case.

The behavior of the several vitamins of the B group in the varied physiological processes which have been discussed are summarized in Table XX. It should be borne in mind, however, that the physiological roles of the B vitamins now recognized may well constitute only a small part of the total number of ways in which these compounds contribute to the integration of plant growth.

REFERENCES

- Addicott, F. T. 1939. *Botan. Gaz.* **100**, 836-843.
Addicott, F. T. 1941. *Botan. Gaz.* **102**, 576-581.
Addicott, F. T. 1942. University of California, unpublished.
Addicott, F. T., and Bonner, J. 1938. *Science* **88**, 577-578.
Addicott, F. T., and Devirian, P. S. 1939. *Am. J. Botany* **26**, 667-671.
Ahlgren, G. H. 1941. *J. Am. Soc. Agron.* **33**, 572-576.
Ahlgren, G. H. 1942. *N. J. Agr. Expt. Sta. Bull.* No. 692, 1-19.
Albaum, H. G., and Cohen, P. P. 1943. *J. Biol. Chem.* **149**, 19-27.
Arnon, D. I. 1940. *Science* **92**, 264-266.
Avery, G. S., Jr. 1935. *Bull. Torrey Botan. Club* **62**, 313-330.
Bartlett, G. 1942. Thesis, University of Chicago.
Beadle, G. W., Mitchell, H. K., and Nyc, J. F. 1947. *Proc. Natl. Acad. Sci.* **33**, 155-158.

- Berger, J., and Avery, G. S., Jr. 1944. *Am. J. Botany* **31**, 11-19.
- Bohonos, N., Hutchings, B. L., and Peterson, W. H. 1941. *J. Bact.* **41**, 40.
- Bonner, D. M. 1940. Thesis, California Institute of Technology.
- Bonner, D. M., and Bonner, J. 1938. *Proc. Natl. Acad. Sci.* **24**, 70-75.
- Bonner, D. M., and Bonner, J. 1940. *Am. J. Botany* **27**, 38-42.
- Bonner, D. M., Haagen-Smit, A. J., and Went, F. W. 1939. *Botan. Gaz.* **101**, 128-144.
- Bonner, J. 1937. *Science* **85**, 183-184.
- Bonner, J. 1938a. *Am. J. Botany* **25**, 543-549.
- Bonner, J. 1938b. *Plant. Physiol.* **13**, 865-868.
- Bonner, J. 1940a. California Institute of Technology, unpublished.
- Bonner, J. 1940b. California Institute of Technology, unpublished.
- Bonner, J. 1940c. California Institute of Technology, unpublished.
- Bonner, J. 1940d. *Plant. Physiol.* **15**, 553-557.
- Bonner, J. 1940e. *Am. J. Botany* **27**, 692-701.
- Bonner, J. 1941. California Institute of Technology, unpublished.
- Bonner, J. 1942a. California Institute of Technology, unpublished.
- Bonner, J. 1942b. California Institute of Technology, unpublished.
- Bonner, J. 1942c. *Bull. Torrey Botan. Club* **69**, 130-133.
- Bonner, J. 1942d. *Botan. Gaz.* **103**, 581-585.
- Bonner, J. 1942e. *Am. J. Botany* **29**, 136-142.
- Bonner, J. 1942f. *Proc. Natl. Acad. Sci.* **28**, 321-324.
- Bonner, J. 1943a. *Bull. Torrey Botan. Club* **70**, 184-189.
- Bonner, J. 1943b. *Botan. Gaz.* **104**, 475-479.
- Bonner, J. 1945. California Institute of Technology, unpublished.
- Bonner, J., and Addicott, F. T. 1937. *Botan. Gaz.* **99**, 144-170.
- Bonner, J., and Axtman, G. 1937. *Proc. Natl. Acad. Sci.* **23**, 453-457.
- Bonner, J., and Buchman, E. R. 1938. *Proc. Natl. Acad. Sci.* **24**, 431-438.
- Bonner, J., and Buchman, E. R. 1939a. California Institute of Technology, unpublished.
- Bonner, J., and Buchman, E. R. 1939b. *Proc. Natl. Acad. Sci.* **25**, 164-171.
- Bonner, J., and Devirian, P. S. 1939. *Am. J. Botany* **26**, 661-665.
- Bonner, J., and Dorland, R. 1943a. *Am. J. Botany* **30**, 414-418.
- Bonner, J., and Dorland, R. 1943b. *Arch. Biochem.* **2**, 451-462.
- Bonner, J., and Erickson, J. 1938. *Am. J. Botany* **25**, 685-692.
- Bonner, J., and Greene, J. 1938. *Botan. Gaz.* **100**, 226-237.
- Bonner, J., and Greene, J. 1939. *Botan. Gaz.* **101**, 491-500.
- Bonner, J., and Wildman, S. G. 1946. *Arch. Biochem.* **10**, 497-518.
- Borgström, G. 1939. *Botan. Notiser* 207-220.
- Bouillenne, R., and Roubaix, J. de. 1942. *Bull. soc. roy. sci. Liège* **11**, 656-675.
- Braunstein, A. E., and Kritsman, M. G. 1937. *Enzymologia* **2**, 129-146.
- Brink, R. A., Cooper, D. C., and Ausherman, L. E. 1944. *J. Heredity* **35**, 67-75.
- Buchman, E. R., and Richardson, E. M. 1939. *J. Am. Chem. Soc.* **61**, 891-896.
- Bunting, A. H., and James, W. O. 1941. *New Phytologist* **40**, 262-267.
- Burgeff, H. 1934. *Ber. deut. botan. Ges.* **52**, 384-390.
- Burkholder, P. R., and McVeigh, I. 1940. *Am. J. Botany* **27**, 853-861.
- Burkholder, P. R., and McVeigh, I. 1942. *Proc. Natl. Acad. Sci.* **28**, 440-446.
- Burkholder, P. R., and McVeigh, I. 1945a. *Plant Physiol.* **20**, 76-82.
- Burkholder, P. R., and McVeigh, I. 1945b. *Plant Physiol.* **20**, 301-306.
- Burkholder, P. R., and Snow, A. G., Jr. 1942. *Bull. Torrey Botan. Club* **69**, 421-428.

- Cheldelin, V. H., Hoag, E. H., and Sarett, H. P. 1945. *J. Bact.* **49**, 41-45.
- Cheldelin, V. H., and Lane, R. L. 1943. *Proc. Soc. Exptl. Biol. Med.* **54**, 53-55.
- Cheldelin, V. H., and Williams, R. J. 1942. *Univ. Texas Pub. No.* **4237**, 105-124.
- Clark, D. G. 1942. *Plant Physiol.* **17**, 137-140.
- Cooper, W. C. 1938. *Botan. Gaz.* **99**, 599-614.
- Cooperative experiment, final report. 1941. California Institute of Technology, mimeographed.
- Cravioto, B. R., Lockhart, E. E., Anderson, R. K., Miranda, F. de P., Harris, R. S., Aguilar, E., Tapia, E. W., Lockhart, H. S., Nutter, M. K., and Guild, L. P. 1945. *J. Nutrition* **29**, 317-329.
- Dennison, R. 1940. *Science* **92**, 17.
- DeRopp, R. S. 1945. *Ann. Botany [N.S.]* **9**, 369-381.
- DeRopp, R. S. 1947. *Am. J. Botany* **34**, 53-62.
- Deuber, C. G. 1940. *Trans. Conn. Acad. Arts Sci.* **34**, 1-83.
- Donnelly, M. 1941. *Science* **94**, 71-72.
- Dorfman, A., Koser, S. A., Reames, H. R., Swingle, K. F., and Saunders, F. 1939. *J. Infectious Diseases* **65**, 163-182.
- Dorfman, A., Koser, S. A., and Saunders, F. 1938. *J. Am. Chem. Soc.* **60**, 2004-2005.
- Eakin, R. E., and Eakin, E. A. 1942. *Science* **96**, 187-188.
- Fiedler, H. 1936. *Z. Botan.* **30**, 385-436.
- Fosse, R. 1926. *Compt. rend.* **182**, 869-871.
- Fosse, R., Graeve, P. de, and Thomas, P. E. 1932. *Compt. rend.* **194**, 1408-1413.
- Fowells, H. A. 1943. *J. Forestry* **41**, 685-686.
- Fraps, G. S., and Fudge, J. F. 1942. *Texas Agr. Expt. Sta. Circ.* **95**, 3-16.
- Gale, E. F., and Epps, H. M. R. 1944. *Biochem. J.* **38**, 232-242.
- Galston, A. W. 1947. *J. Biol. Chem.* **169**, 465-466.
- Gautheret, R. J. 1946. *Growth Supplement*, 21-43.
- Geddes, W. F., and Levine, M. N. 1942. *Cereal Chem.* **19**, 547-552.
- Gisiger, L. 1943. *Mitt. Lebensm. Hyg.* **34**, 315-317.
- Gollub, M., and Vennesland, B. 1947. *J. Biol. Chem.* **169**, 233-234.
- Gorham, P. R. 1941. *Am. J. Botany* **28**, 98-101.
- Gorham, P. R. 1945. *Am. J. Botany* **32**, 496-505.
- Gregory, F. G. 1928. *Ann. Botany* **42**, 469-507.
- Gunsalus, I. C., and Bellamy, W. D. 1944a. *J. Biol. Chem.* **155**, 357-358.
- Gunsalus, I. C., and Bellamy, W. D. 1944b. *J. Biol. Chem.* **155**, 557-563.
- Gunsalus, I. C., Bellamy, W. D., and Umbreit, W. W. 1944. *J. Biol. Chem.* **155**, 685-686.
- Haber, E. S., and Edgecombe, S. W. 1940. *Trans. Iowa State Hort. Soc.* **75**, 142-153.
- Hamner, C. L. 1940. *Botan. Gaz.* **102**, 156-168.
- Hildebrandt, A. C., Riker, A. J., and Duggar, B. M. 1946. *Cancer Research* **6**, 368-377.
- Hinton, J. J. C. 1942. *J. Soc. Chem. Ind.* **61**, 143-144.
- Hinton, J. J. C. 1944. *Biochem. J.* **38**, 214-217.
- Hitchcock, A. E., and Zimmerman, P. W. 1940. *Contribs. Boyce Thompson Inst.* **11**, 143-160.
- Hitchcock, A. E., and Zimmerman, P. W. 1941. *Contribs. Boyce Thompson Inst.* **12**, 143-156.
- Hoffer, A., Alcock, A. W., and Geddes, W. F. 1946. *Cereal Chem.* **23**, 76-83.
- Horowitz, N. H., and Heegaard, E. 1941. *J. Biol. Chem.* **137**, 475-483.

- Knight, B. C. J. G. 1937a. *Biochem. J.* **31**, 731-737.
- Knight, B. C. J. G. 1937b. *Biochem. J.* **31**, 966-973.
- Kögl, F., and Haagen-Smit, A. J. 1936. *Z. Physiol. Chem.* **243**, 209-226.
- Kohler, G. O. 1944. *J. Biol. Chem.* **152**, 215-223.
- Kramer, A., and Schrader, A. L. 1942. *J. Agr. Research* **65**, 313-328.
- Landy, M. 1938. *Proc. Soc. Exptl. Biol. Med.* **38**, 504-506.
- LaRue, C. D. 1936. *Bull. Torrey Botan. Club* **63**, 365-382.
- Laurie, A., and Kiplinger, D. C. 1941. *Ohio Agr. Expt. Sta. Bull.* No. **26**, 17-28.
- Levine, M., and Lein, J. 1941. *Am. J. Botany* **28**, 163-168.
- Lichstein, H. C., Gunsalus, I. C., and Umbreit, W. W. 1945. *J. Biol. Chem.* **161**, 311-320.
- Lichstein, H. C., and Umbreit, W. W. 1947a. *J. Biol. Chem.* **170**, 329-336.
- Lichstein, H. C., and Umbreit, W. W. 1947b. *J. Biol. Chem.* **170**, 423-424.
- Lipmann, F. 1937. *Enzymologia* **4**, 65-72.
- Lipmann, F. 1939. *Cold Spring Harbor Symposia Quant. Biol.* **7**, 248-259.
- Lipmann, F. 1941. *Advances in Enzymol.* **1**, 99-162.
- Lipmann, F., Kaplan, N. O., Novelli, G. D., Tuttle, L. C., and Guirard, B. M. 1947. *J. Biol. Chem.* **167**, 869.
- Lockhart, E. E. 1939. *Biochem. J.* **33**, 613-617.
- Lohman, K., and Schuster, P. 1937. *Naturwissenschaften* **25**, 26-27.
- Loo, S. W. 1944. Report to Smith College Embryo Culture Conference.
- Loo, S. W. 1945. *Am. J. Botany* **32**, 13-17.
- Lundegårdh, H., and Wikén, B. 1943. *Kgl. Lantbruksakad. Tid.* **82**, 99-122.
- Lwoff, A., and Lwoff, M. 1937a. *Proc. Roy. Soc. London* **B122**, 352-359.
- Lwoff, A., and Lwoff, M. 1937b. *Proc. Roy. Soc. London* **B122**, 360-373.
- McClary, J. E. 1940. *Proc. Natl. Acad. Sci.* **26**, 581-587.
- McVeigh, I. 1944. *Bull. Torrey Botan. Club* **71**, 438-444.
- Meyer, J. R. 1944. *Rev. Applied Mycol.* **23**, 403.
- Miller, D. 1940. California Institute of Technology, unpublished.
- Minarik, C. E. 1942. *Plant Physiol.* **17**, 141-142.
- Minnun, E. C. 1941a. *Proc. Am. Soc. Hort. Sci.* **38**, 475-476.
- Minnun, E. C. 1941b. *Botan. Gaz.* **103**, 397-400.
- Morel, G. 1946. *Compt. rend.* **223**, 166-168.
- Murneek, A. E. 1941. *Proc. Am. Soc. Hort. Sci.* **38**, 715-717.
- Myers, H. E., Jugenheimer, R. W., and Heyne, E. G. 1941. *J. Am. Soc. Agron.* **33**, 474-476.
- Neipp, L. 1943. *Hundert Jahre Schweiz. Apoth. Ver.* 1843-1943, 510-520.
- Nobécourt, P. 1940. *Compt. rend. soc. biol.* **133**, 530-532.
- Nobécourt, P. 1943. *Rev. sci.* **81**, 161-170.
- Nobécourt, P., and Kofler, L. 1945. *Compt. rend.* **221**, 53-54.
- Noggle, G. R., and Wynd, F. L. 1943. *Botan. Gaz.* **104**, 455-459.
- Novelli, G. D., and Lipmann, F. 1947. *Arch. Biochem.* **14**, 23-27.
- Ochoa, S., and Weisz-Tabori, E. 1945. *J. Biol. Chem.* **159**, 245-246.
- Okunuki, K. 1937. *Acta Phytochim. Japan* **9**, 267-285.
- Okunuki, K. 1940. *Acta Phytochim. Japan* **11**, 249-260.
- Ondratschek, K. 1940a. *Arch. Mikrobiol.* **11**, 89-117.
- Ondratschek, K. 1940b. *Arch. Mikrobiol.* **11**, 219-226.
- Ondratschek, K. 1940c. *Arch. Mikrobiol.* **11**, 228-238.
- Ondratschek, K. 1940d. *Arch. Mikrobiol.* **11**, 239-263.
- Ondratschek, K. 1941a. *Arch. Mikrobiol.* **12**, 46-57.

- Ondratschek, K. 1941b. *Arch. Mikrobiol.* **12**, 229-240.
- Onslow, M. W. 1931. *The Principles of Plant Biochemistry*. Part I. Cambridge University Press, London.
- Parker, E. R., Turrell, F. M., and Bonner, J. 1941. *Proc. Am. Soc. Hort. Sci.* **38**, 49-58.
- Pearse, H. L. 1939. *Ann. Rept. East Malling Research Sta. Kent* **27**, 157-166.
- Pulkki, L. H., and Puutula, K. 1941. *Biochem. Z.* **308**, 122-127.
- Rautanen, N. 1946. *J. Biol. Chem.* **163**, 687-688.
- Reeves, A. M. 1942. *Gladiolus* **17**, 141-145.
- Robbins, W. J. 1922a. *Botan. Gaz.* **73**, 376-90.
- Robbins, W. J. 1922b. *Botan. Gaz.* **74**, 59-79.
- Robbins, W. J. 1940. *Plant Physiol.* **15**, 547-552.
- Robbins, W. J. 1941. *Am. J. Bot.* **28**, 216-225.
- Robbins, W. J. 1942. *Am. J. Bot.* **29**, 241-245.
- Robbins, W. J., and Bartley, M. A. 1937a. *Science* **85**, 246-247.
- Robbins, W. J., and Bartley, M. A. 1937b. *Proc. Natl. Acad. Sci. U.S.* **23**, 385-388.
- Robbins, W. J., and Kavanagh, F. 1938a. *Proc. Natl. Acad. Sci. U.S.* **24**, 141-145.
- Robbins, W. J., and Kavanagh, F. 1938b. *Proc. Natl. Acad. Sci. U.S.* **24**, 145-147.
- Robbins, W. J., and Kavanagh, F. 1938c. *Proc. Natl. Acad. Sci. U.S.* **24**, 229-230.
- Robbins, W. J., and Schmidt, M. B. 1939a. *Proc. Natl. Acad. Sci. U.S.* **25**, 1-3.
- Robbins, W. J., and Schmidt, M. B. 1939b. *Am. J. Botany* **26**, 149-159.
- Robbins, W. J., and Schmidt, M. B. 1939c. *Bull. Torrey Botan. Club* **66**, 193-200.
- Rosen, F., Huff, J. W., and Perlzweig, W. A. 1946. *J. Biol. Chem.* **163**, 343-344.
- Rytz, W. von, Jr. 1939. *Ber. schweiz. botan. Ges.* **49**, 339-399.
- Schaffstein, G. 1938. *Jahrb. wiss. Botan.* **86**, 720-752.
- Schales, O., Mims, V., and Schales, S. S. 1946. *Arch. Biochem.* **10**, 455-465.
- Schittenhelm, A. Z. 1909. *Z. physiol. Chem.* **63**, 289.
- Schopfer, W. H. 1934. *Arch. Mikrobiol.* **5**, 511-549.
- Skoog, F. 1944. *Am. J. Botany* **31**, 19-24.
- Snell, E. E., Guirard, B. M., and Williams, R. J. 1942. *J. Biol. Chem.* **143**, 519-530.
- Somers, G. F., and Coolidge, M. H. 1945. *Science* **101**, 98-99.
- Somers, G. F., Coolidge, M. H., and Hamner, K. C. 1945. *Cereal Chem.* **22**, 333-340.
- Sonne, J. C., Buchanan, J. M., and Delluva, A. M. 1946. *J. Biol. Chem.* **166**, 395-396.
- Stoutemyer, V. T. 1940. *Am. Nurseryman* **71**, 11-13, 32-33.
- Sukhorukov, K., and Filippov, V. 1940. *Compt. rend. acad. sci. U.R.S.S.* **29**, 347-350.
- Swartz, D. B. 1941. *Botan. Gaz.* **103**, 366-373.
- Tatum, E. L. 1945. *J. Biol. Chem.* **160**, 455-459.
- Tatum, E. L., and Beadle, G. W. 1942. *Proc. Natl. Acad. Sci.* **28**, 234-243.
- Tatum, E. L., and Bell, T. T. 1946. *Am. J. Botany* **33**, 15-20.
- Tauber, H. 1937. *Proc. Soc. Exptl. Biol. Med.* **37**, 541-543.
- Templeman, W. G., and Marmoy, C. J. 1940. *Ann. Applied Biol.* **27**, 453-471.
- Templeman, W. G., and Pollard, N. 1941. *Ann. Botany [N.S.]* **5**, 133-147.
- Thimann, K. V., and Delisle, A. L. 1939. *J. Arnold Arboretum Harvard Univ.* **20**, 116-231.
- Tincker, M. A. H., and Unwin, C. H. 1939. *J. Roy. Hort. Soc.* **64**, 554-566.
- Tukey, H. B. 1934. *Proc. Am. Soc. Hort. Sci.* **32**, 313-322.
- Tukey, H. B., and Brase, K. D. 1941. *Proc. Am. Soc. Hort. Sci.* **38**, 339-340.

- Umbreit, W. W., and Gunsalus, I. C. 1945. *J. Biol. Chem.* **159**, 333-341.
- Underkofler, L. A., Bantz, A. C., and Peterson, W. H. 1943. *J. Bact.* **45**, 183-190.
- Unna, K. 1940. *Proc. Soc. Exptl. Biol. Med.* **43**, 122-124.
- Van Overbeek, J. 1940. *Proc. Natl. Acad. Sci. U.S.* **26**, 441-443.
- Van Overbeek, J., Conklin, M. E., and Blakeslee, A. F. 1941. *Science* **94**, 350-351.
- Vennesland, B., and Felsner, R. Z. 1946. *Arch. Biochem.* **11**, 279-306.
- Vigneaud, V. du, Dittmer, K., Hague, E., and Long, B. 1942. *Science* **96**, 186-187.
- Virtanen, A. I., and Laine, T. 1937. *Enzymologia* **3**, 266-270.
- Virtanen, A. I., and Laine, T. 1941. *Biochem. Z.* **308**, 213-215.
- Vivino, A. E., and Palmer, L. S. 1944. *Arch. Biochem.* **4**, 129-136.
- Vyvyany, M. C. 1924. *Ann. Botany* **38**, 60-103.
- Warburg, O., and Christian, W. 1932. *Naturwissenschaften* **20**, 980-981.
- Ward, A. H. 1943. *Chemistry and Industry* 11-14.
- Warner, G. C., and Went, F. W. 1939. Rooting of Cuttings with Indoleacetic Acid and Vitamin B₁. Plant Culture Publishing Co., Pasadena, Calif.
- Weevers, Th. 1930. *Arch. Néerl. Sci.* **5**, 111-195.
- Weevers, Th. 1932. *Rec. trav. Botan. néerland.* **30**, 336-462.
- Went, F. W. 1932. *Jahrb. wiss. Bot.* **76**, 528-557.
- Went, F. W. 1938a. *Plant Physiol.* **13**, 55-80.
- Went, F. W. 1938b. *Am. J. Botany* **25**, 44-55.
- Went, F. W., and Bonner, D. 1943. *Arch. Biochem.* **1**, 439-452.
- Went, F. W., Bonner, J., and Warner, G. C. 1938. *Science* **87**, 170-171.
- Went, F. W., and Thimann, K. V. 1937. *Phytohormones*. Macmillan, New York.
- West, P. M. 1939. *Nature* **144**, 1050-1051.
- White, P. R. 1932a. *Plant Physiol.* **7**, 613-628.
- White, P. R. 1932b. *Arch. expl. Zellforsch. Gewebezücht.* **12**, 602-620.
- White, P. R. 1934. *Plant Physiol.* **9**, 585-600.
- White, P. R. 1937. *Plant Physiol.* **12**, 803-811.
- White, P. R. 1939a. *Am. J. Botany* **26**, 59-64.
- White, P. R. 1939b. *Plant Physiol.* **14**, 527-538.
- White, P. R. 1943. *Am. J. Botany* **30**, 33-36.
- William, A. 1939. *Sucr. belge* **58**, 461-478.
- Williams, R. J., and Rohrmann, E. 1936. *J. Am. Chem. Soc.* **58**, 695.
- Woodhouse, W. W., Jr., and Morris, H. D. 1942. *J. Am. Soc. Agron.* **34**, 322-326.
- Woolley, D. W., Strong, F. M., Madden, R. J., and Elvehjem, C. A. 1938. *J. Biol. Chem.* **124**, 715-723.
- Zopf, L. C. 1940. *J. Am. Pharm. Assoc. Sci. Ed.* **29**, 487-496.

The Influence of the Adrenal Cortex on the Metabolism of Water and Electrolytes

By EDWARD C. KENDALL

Division of Biochemistry, Mayo Foundation, Rochester, Minnesota

CONTENTS

	<i>Page</i>
I. Introduction.....	278
II. Survival after Adrenalectomy.....	279
III. Normal Distribution of Electrolytes.....	281
1. Retention.....	281
2. Stability of Equilibria of Blood.....	281
3. Intracellular and Extracellular Compartments.....	281
Sodium and Chloride	282
Potassium.....	282
4. Functional Changes.....	283
Asphyxia, the Tension of Carbon Dioxide, Rate of Blood Flow ...	283
Glucose and Other Metabolites.....	283
Epinephrine.....	283
Contraction of Muscle.....	283
5. The Distribution of Water.....	284
IV. Abnormal Distribution of Electrolytes and Water.....	284
1. After Adrenalectomy.....	284
Sodium, Chloride, and Water.....	284
Potassium.....	285
2. After Intraperitoneal Injection of Glucose.....	285
3. After Water Intoxication.....	286
V. Renal Function.....	286
VI. Renal Function after Adrenalectomy.....	287
1. The Excretion of Sodium and Chloride	287
2. The Excretion of Potassium.....	288
3. Glomerular and Tubular Activity.....	288
4. The Excretion of Water.....	289
VII. The Transfer of Water and Electrolytes through the Intestinal Wall....	290
VIII. The Relation between Sodium Salts and the Hormones of the Adrenal Cortex.....	292
IX. The Influence of Sodium Chloride on Physiologic Processes.....	293
1. Renal Functions.....	293
Water.....	293
Sodium and Potassium.....	293
Excretion of Urea.....	294
2. Intestinal Absorption.....	295
Electrolytes.....	295

	<i>Page</i>
Glucose.....	295
Fat.....	296
3. Metabolism of Protein.....	296
4. Metabolism of Carbohydrate.....	298
Deposition of Glycogen in the Liver.....	298
Gluconeogenesis.....	299
5. Metabolism of Fat.....	301
6. Metabolism of Potassium.....	302
7. Miscellaneous Effects.....	303
Appetite.....	303
Spontaneous Activity and Response to Stimulation of Muscle....	303
Toxicity of Certain Drugs and Salts.....	304
Intestinal Response to Vasoconstrictor Influences.....	304
Metabolism of Melanin.....	305
Changes in the Anterior Pituitary Body.....	305
The Estrous Cycle and Reproduction.....	305
Erythropoiesis.....	305
Basal Metabolism and Uptake of Oxygen.....	306
X. Symptoms of Adrenal Insufficiency.....	306
1. Sodium, Chloride, and Water.....	306
2. Potassium.....	307
Muscle.....	308
Heart.....	308
Toxicity.....	309
XI. Qualitative Differences of Steroids.....	312
1. The Sodium Factor.....	312
2. The Amorphous Fraction.....	313
3. Estrogens, Androgens, and Related Compounds.....	313
4. Desoxycorticosterone.....	314
Sodium and Chloride.....	314
Potassium.....	315
5. Corticosterone and Related Compounds.....	316
XII. Electrolytes and Adrenal Insufficiency in Clinical Medicine.....	316
1. Diagnosis.....	316
2. Treatment with Control of Electrolytes.....	317
3. Treatment with Desoxycorticosterone Acetate.....	318
XIII. Comment and Summary.....	319
References.....	321

I. INTRODUCTION

It must be remembered, however, that when nothing was known of the function of the thyroid or of the internal secretion of the pancreas a favorite assumption was that the serious consequences of their removal were due to the loss of a "detoxicating" action, in the absence of which toxic substances which could no longer be neutralized, accumulated in the body. As soon as it was established that thyroid "substitution therapy" was a brilliant success, especially when the specific hormone of the thyroid was isolated, nobody talked any more of the detoxicating function of the gland. It is the same now with the pancreas. It was the same for a time with the adrenals after the discovery of epinephrin. When it was seen, after the first enthusiasm had passed,

that the discovery of epinephrin had not in any important degree solved the riddle of adrenal function, the ghost of the "detoxication" theory began to walk again.—G. N. STEWART (1924).

For several years after Stewart expressed these thoughts in 1924 the ghost of the detoxication theory continued to walk. The observation of Stewart and Rogoff (1925), Rogoff and Stewart (1928) and Corey (1927) that infusion of large volumes of physiologic salt solution or Ringer's solution produced an almost specific effect on the condition of adrenalectomized dogs was first interpreted as a washing out of toxic substances. However, as more detailed chemical studies were carried out it became evident that the distribution and excretion of water and of sodium, potassium and chloride ions were disturbed. The nature of the changes produced by adrenalectomy on the metabolism of sodium, potassium, and chloride and the influence of the adrenal cortex on physiologic processes are more closely examined in the following pages. The adrenal cortex has now joined the thyroid, the pancreas, and the adrenal medulla. The ghost of the detoxication theory walks no more.

II. SURVIVAL AFTER ADRENALECTOMY

The phenomenon that all investigators encountered after adrenalectomy of experimental animals was the development of a condition that Banting and Gairns (1926) characterized for dogs that survived less than 50 hours by rapid pulse, elevated temperature, thirst, loss of appetite, terminal fall of blood sugar, restlessness, suppression of urine, salivation, vomiting, diarrhea, unconsciousness, coma, and convulsions.

The fatal termination in dogs surviving more than 100 hours may begin with suppression of urine, loss of appetite, weakness, especially in the hind legs, drowsiness, and dilatation of the pupils. The superficial veins of the legs collapse and samples of blood are obtained with difficulty. The blood becomes thick and dark. Finally, within a few hours of death there may be vomiting and diarrhea.

Chemical studies on the constituents of the blood after adrenalectomy in the dog revealed a decrease of chloride ion (Lucas, 1926) but Banting and Gairns were not able to prevent death by administration of sodium chloride even when the urea, nonprotein nitrogen, and chloride were maintained at almost normal concentrations.

Marine and Baumann (1927) observed that injections of a solution of sodium chloride produced an increase in the time of survival of adrenalectomized cats, and Baumann and Kurland (1927) added two other essential facts. Associated with the loss of chloride there was a decrease of sodium and an increase of potassium in the serum.

Loeb (1932, 1933) made the outstanding contribution that in patients

who had Addison's disease the concentrations of sodium and chloride in the blood were lower than normal and that there was a striking improvement of the clinical condition when an adequate amount of sodium chloride was given in the diet. A recurrence of the symptoms of severe adrenal insufficiency resulted within 48 hours after sodium chloride was withheld.

Metabolic studies with experimental animals (Loeb *et al.*, 1933) confirmed the observation on the loss of sodium and chloride from the plasma, and it was also found by these same investigators that in dogs the decrease of the content of sodium was more rapid than that of chloride. To control the condition of acidosis which developed because of the greater loss of sodium than chloride Harrop *et al.* (1935) administered sodium bicarbonate with sodium chloride by stomach tube. This treatment maintained adrenalectomized dogs indefinitely without the use of an extract of the adrenal cortex. The concentrations of the constituents of the blood were all within the normal limits except for potassium.

Contemporary with this work it was shown that the high concentration of potassium in the serum could be reduced to normal if the intake of sodium chloride was high and that of potassium was low (Allers, 1935; Allers *et al.*, 1936; Allers and Kendall, 1937). Such a diet was based on the previous work which has been described, and that of Hastings and Compere (1930) which had demonstrated the elevation of potassium in the serum after adrenalectomy.

With the regimen used by Allers development of acidosis was checked with sodium citrate in place of sodium bicarbonate, since the former was better tolerated, and the sodium salts were added to the diet and drinking water. There were two reasons for this method of administration: it involved the least labor, and it seemed desirable to extend the intake of sodium chloride as far as possible throughout the 24 hours of each day. It is difficult to imagine a more simple answer to a complicated and important problem. That the answer was correct has been shown repeatedly.

A word of explanation is necessary to answer some obvious questions. Banting and Gairns (1926), Rogoff and Stewart (1928), Swingle *et al.* (1934a), Zwemer (1934), Loeb *et al.* (1935), and Silvette and Britton (1935) concluded that after adrenalectomy the administration of sodium chloride was helpful but that the survival time was only moderately prolonged. There are many different reasons for this consensus but all of these reasons have one thing in common; that is, they were all based on investigations which failed to satisfy the rather narrow limits of the necessary experimental conditions. Before these conditions had been established there was no known good reason why it was not possible to

furnish food that would be relished and eagerly eaten. With dogs and cats this was meat, and almost invariably in the early stages of the investigation the diets contained a large proportion of this foodstuff (Marine and Baumann, 1927; Loeb *et al.*, 1933; Harrop, 1936a; Harrop *et al.*, 1936). This raised the daily intake of potassium and, as a consequence, the severity of the symptoms was increased, the appetite was impaired, all food except meat was refused, and thus a vicious cycle was produced. It is clear that the eating habits of the animals, the diet, the manner of administration, and the amount and type of sodium salts provided were important factors which shaped the early course of the experimental work.

When it was recognized that the critical condition produced by adrenalectomy was associated with dehydration and an abnormal distribution of water and electrolytes (Loeb *et al.*, 1933) investigations were carried out to determine the extent and cause of these changes. These inquiries, together with other studies of the metabolism of electrolytes, provided quantitative data which are essential for correlation with the functional changes observed.

III. NORMAL DISTRIBUTION OF ELECTROLYTES

1. Retention of Electrolytes

The intact animal and man can retain sodium, potassium, and chloride over long intervals of time even when the diet contains but small amounts of these ions. This is true whether sodium, potassium, or chloride is individually excluded from the diet or the diet is low in all minerals (Brooke and Smith, 1933; Orent-Keiles *et al.*, 1937; Orent-Keiles and McCollum, 1940, 1941; Greenberg and Cuthbertson, 1942; Cuthbertson and Greenberg, 1945).

2. Stability of Electrolyte Equilibria in Blood and Tissues

Although the diet may be extremely unfavorable, the animal organism is able to maintain this vital balance with little alteration for a relatively long period (Smith and Smith, 1934; Light *et al.*, 1934). A similar resistance to change is shown with a diet which has an unusually high content of sodium chloride and other salts. Marked changes take place only when death approaches and in all probability these changes are responsible for death (Heller and Paul, 1934).

3. Intracellular and Extracellular Compartments

Manery and Hastings (1939) pointed out that muscle cells (that is, the intracellular phase of the tissue) contain a high concentration of

potassium and phosphate, little if any sodium, and no chloride. The remainder, called the extracellular phase, approximates a serum ultrafiltrate in composition and contains all of the chloride and most of the sodium.

As a first approximation Manery and Hastings also concluded (1) that the electrolytes in the extracellular fluid of all tissues are essentially identical, (2) that the mass of the extracellular phase varies from one type of tissue to another, and (3) that tissues have varying proportions of at least two types of cells: those which contain no sodium or chloride, and those which contain sodium and chloride.

The tissues which have a large proportion of chloride-containing cells are blood, connective tissue, gastric mucosa, testes, and probably lung.

Sodium and Chloride. Muntwyler *et al.* (1940b) found the content of sodium and chloride of normal dog muscle to be approximately 28 mM and 20 mM respectively per 100 g. of fat-free tissue.

When radioactive sodium was injected into experimental animals it rapidly permeated all fluids and tissues of the body where sodium is normally present (Manery and Bale, 1941; Greenberg *et al.*, 1940). The results confirmed earlier conclusions that sodium is essentially an extracellular ion. In rats maintained on a low content of potassium but with adequate sodium in the diet the potassium in the muscle was in part replaced by sodium. Radioactive sodium penetrated the muscles of these rats in proportion to the sodium already present (Heppel, 1940). Manery and Bale (1941) and Manery and Haege (1941) found that the radioactive isotopes of sodium and chloride rapidly penetrated the tissue of skin, kidney, liver, muscle, and tendon and were distributed between the tissue and plasma to the same magnitude as the normal isotopes. It was suggested that a comparison of the unstable isotopes of sodium and chloride allows a more reasonable measurement of the extracellular water of many tissues than has hitherto been possible.

Potassium. Miller and Darrow (1940a) found that the potassium in the muscle of rats was usually 47–49 mM/100 g. of fat-free solids but may decrease to as low as 44 mM as a result of a diet with high content of potassium or after an interval of 18 hours following an injection of potassium chloride or of sodium chloride with sodium bicarbonate. Per kilogram of intracellular water, the potassium in the muscle of rats apparently can vary from 146 to 160 mM without a change in the sodium content of the serum. Miller and Darrow further showed that when the potassium in serum is raised by injection of potassium salts the concentration of this cation in the muscle increases quickly to 50 or 53 mM/100 g. of fat-free solids but returns to normal within 90 minutes.

A salt of potassium injected intravenously into dogs was distributed

through a volume of fluid which was much greater than that of the extracellular compartment and corresponded more nearly to that of the total fluid of the body. This indicated that exogenous potassium entered some, and probably most, of the cells of the body (Winkler and Smith, 1938; Houssay and Marenzi, 1937).

The distribution of radioactive potassium confirmed this conclusion (Joseph *et al.*, 1939; Noonan *et al.*, 1941) but indicated further that an excess of potassium in the plasma was probably quickly removed by the viscera and was slowly released to the muscles (Fenn *et al.*, 1941).

4. Functional Changes

Asphyxia, the Tension of Carbon Dioxide, Rate of Blood Flow. Asphyxia for 4 or 5 minutes results in a marked rise of serum potassium. A temporary rise followed breathing a mixture of 10% carbon dioxide and 90% oxygen (Cattell and Civin, 1938), and *in vitro* studies showed that an increase of the tension of carbon dioxide tended to cause potassium to shift from plasma to the muscle (Fenn and Cobb, 1935). Reduction of the rate of flow of blood caused a rapid increase of potassium in the plasma. The source of the potassium appeared to be the muscle or liver (Baetjer, 1935; Mullin *et al.*, 1938; Fenn *et al.*, 1939).

Glucose and Other Metabolites. Potassium leaves the plasma and enters the intracellular compartment after the intravenous injection of glucose, levulose, or pyruvic acid (Flock *et al.*, 1938). A similar change of potassium from solution to cells has been shown for suspended erythrocytes (Danowski, 1941; Harris, 1941) and for yeast in the presence of glucose (Pulver and Verzár, 1940).

Epinephrine. The administration of epinephrine produces a rapid increase of the concentration of potassium in the plasma (D'Silva, 1934; Brewer *et al.*, 1939), and this in turn is followed by a decrease to levels below the normal value (Keys, 1938; Castleden, 1938; Larson, 1940; Stickney, 1941). Perfusion of the liver with a saline solution which contained epinephrine released potassium (D'Silva, 1936).

Contraction of Muscle. When muscle is made to contract by injection of acetylcholine, potassium is liberated (Cicardo and Moglia, 1940a; Somogyi and Verzár, 1941). Contraction of muscle after tetanic stimulation likewise results in release of potassium to the plasma and uptake of water and sodium by the muscle (Fenn and Cobb, 1936; Fenn, 1937; Fenn *et al.*, 1938; Wood *et al.*, 1940; Cicardo and Moglia, 1940b). It is probable that the liberated potassium is taken up in part by the liver. In recovery the reverse changes must occur, since it has been shown that muscle regained the potassium which had been lost. This potassium

cycle is comparable, therefore, to the well-known carbohydrate cycle (Fenn, 1939, 1940).

5. *The Distribution of Water*

The prevailing opinion as to the behavior of cell fluids was expressed by Peters (1935) as follows: Cells adjust themselves to variations in osmotic pressure of the fluid environment by interchange of fluid rather than of salts. This adjustment would be followed by changes of the cell volume and by variation of the total cellular fluid of the body.

The concentration of hydrogen ion influences the volume of the extracellular and intracellular phases (Hastings and Eichelberger, 1937). The degree of dehydration determines the extent of the changes in the volume of the plasma and of the extracellular and intracellular phases (Wiley and Wiley, 1933). Both the interstitial fluid and the plasma contribute to the fluid lost from the extracellular compartment during dehydration. However, except in the early stages a much larger amount is contributed by the interstitial fluid than by the plasma (Mellors *et al.*, 1942a, 1942b). Ashworth and Gill (1944) showed that changes of fluid in the intracellular compartment followed changes in the osmotic pressure of the extracellular phase in the direction which was predicted.

Darrow (1944) concluded that water, like the other constituents of the body, is in a dynamic state and that all body water is being constantly exchanged. Water is not fixed within extracellular and intracellular phases. The amount of water which is held in a particular location is controlled by many factors. Among these factors is the distribution of sodium and potassium, both of which are also in a dynamic state in which all parts of the body are accessible. A simple type of osmotic relationship does not describe the behavior of body fluids.

IV. ABNORMAL DISTRIBUTION OF ELECTROLYTES AND WATER

1. *After Adrenalectomy*

Sodium, Chloride, and Water. The early investigation of the relation between the adrenal cortex and the loss of electrolytes was confined to a study of changes in the plasma. These results, to which reference has been made, were confirmed by Harrop and Weinstein (1933), Harrop *et al.* (1933a, 1933b), Zwemer and Sullivan (1934), Silvette (1934), Harrison and Darrow (1938), Darrow *et al.* (1939), Muntwyler *et al.* (1940a) and others.

Muntwyler *et al.* (1940b) concluded that the outstanding changes of skeletal muscles of dogs in adrenal insufficiency were a loss of sodium and a gain of water. Associated with these changes was a tendency for the content of chloride to fall. Helve (1940) confirmed these observations.

Since muscle from an adrenalectomized animal contains more water than normal muscle, exposure to Ringer's solution should result in a smaller uptake of water by the former. Evidence for this has been secured by Ponder and Gaunt (1934) and by Angerer and Angerer (1941).

Potassium. Since the first studies on the distribution of electrolytes after adrenalectomy an increase of potassium in the plasma has been observed consistently. The source of the potassium is the diet, for it is possible to maintain the concentration of potassium within normal limits by restriction of the intake (Allers and Kendall, 1937).

Unless the intake of potassium is limited there is an appreciable increase in the concentration of potassium in the muscle when the result is expressed per unit of solids (Hegnauer and Robinson, 1936; Harrison and Darrow, 1938; Muntwyler *et al.*, 1940b). The high values given for potassium in the tissues of the rat by Buell and Turner (1941) after adrenalectomy have been questioned by Darrow (1944).

Other evidence for an increase of potassium in the tissues is given by studies on the metabolic balance of this cation. Both the amount of potassium retained during the interval of adrenal insufficiency and the amount excreted after treatment with cortical hormone are too large to be contained in the extracellular fluids. In the rat the retention of potassium after adrenalectomy can be accounted for by the increased concentration in muscle cells and in the extracellular fluid (Harrison and Darrow, 1938).

2. After Intraperitoneal Injection of Glucose

In 1934-1935 Darrow and Yannet devised an ingenious method to cause a marked loss of sodium and chloride from the extracellular compartment without a significant change of potassium and without loss of body water. This was accomplished by an intraperitoneal injection of an isotonic solution of glucose. Sodium and chloride rapidly diffused into the solution and this resulted in a shift of water to the intracellular phase which caused concentration of the blood and the usual signs of dehydration.

Gilman (1934), through use of this method, showed that the decrease of the volume of the blood even in the intact animal resulted in a condition which in many respects simulated the prostration which follows adrenalectomy. Withdrawal of 6-7 cc. blood/kilogram of body weight reduced the blood pressure to shock level, but after several hours the dog invariably returned to a normal condition. Water again entered the extracellular compartment, the blood became diluted, and there was an increase of the sodium and chloride in the blood to normal values.

When an isotonic solution of glucose was injected intraperitoneally

into adrenalectomized dogs a change of the distribution of water occurred which was the same as observed in normal dogs. However, the effect was much more profound and the experiment was terminated by death. These dogs lacked the power to dilute the blood and redistribute the fluid held within the tissue cells (Parkins *et al.*, 1935; Swingle *et al.*, 1936a; Harrop, 1936b).

Similar observations have been made on cats, rabbits and rats (Robinson and Hegnauer, 1936; Gaunt *et al.*, 1937; Remington, 1940, 1943).

3. After Water Intoxication

The decrease of the concentration of electrolytes in the plasma which follows the intraperitoneal injection of glucose was obtained with only trivial changes of the total amount of water in the experimental animal. Another method to produce a decrease of sodium and chloride in the extracellular compartment is to administer excessive amounts of water. Eventually this brings about a decrease of the osmotic pressure and entrance of water into the intracellular phase. If this hydration proceeds far enough convulsions and death result (Rowntree, 1926).

Swingle *et al.* (1937a) concluded that if intoxication from excessive amounts of water follows hydration of cells, then in such animals the disturbance of the distribution of water and electrolytes is analogous to that found in adrenalectomized animals after the serum electrolytes have been depleted by an intraperitoneal injection of isotonic solution of glucose. Although administration of water to normal dogs decreased the sodium and chloride of the serum, recovery was spontaneous if administration of water was stopped with the onset of convulsions. When healthy vigorous adrenalectomized dogs maintained with cortical extract were treated in the same manner recovery from the prostration and convulsions did not occur.

Protection was afforded normal rats against otherwise lethal water intoxication by the use of an extract of the adrenal cortex and in the adrenalectomized dog or rat the administration of a hypertonic solution of sodium chloride or the hormones of the adrenal cortex increased the resistance of the animal to water intoxication (Swingle *et al.*, 1941; Eversole *et al.*, 1942; Gaunt, 1944a, 1944b; Gaunt *et al.*, 1944; Hays and Mathieson, 1945).

V. RENAL FUNCTION

A widely accepted concept for the formation of urine by the kidneys is filtration through the glomerulus to yield an ultrafiltrate of plasma

and subsequent reabsorption of electrolytes, glucose, amino acids and other substances together with most of the water in the tubules (Smith, 1937, 1943). The systemic blood pressure is an important factor since the rate of formation of urine, the removal of urea from the blood and filtration through the glomerulus have been shown to vary with the renal blood pressure. The fraction of the plasma which is filtered through the glomerulus depends on the state of constriction or dilatation of the glomerular arterioles, on the permeability of the glomerular capillaries, on the intrarenal or capsular pressure, and perhaps on the period of contact of the blood with the glomerular capillaries (Smith, 1937, 1943).

Tubular reabsorption of urea and electrolytes is modified by the rate of flow of urine, since in case of low rate more time is available for return of solutes from the urine into the blood stream, whether by diffusion (Holten and Rehberg, 1931) or by other mechanism (Shannon, 1936). In addition, there are many examples of a delicately balanced mechanism which is not well defined. The excretion of one substance may influence the reabsorption of another by the tubules. The infusion of hypertonic solutions of sodium and potassium chloride suppressed the reabsorption of ascorbic acid (Selkurt and Houck, 1941).

The excretion of sulfate is somewhat increased by the simultaneous injection of sodium chloride, but excretion of chloride is almost completely repressed by the sulfate ion, even when a large infusion of sodium chloride is given simultaneously (Schwartz *et al.*, 1942).

VI. RENAL FUNCTION AFTER ADRENALECTOMY

1. *The Excretion of Sodium and Chloride*

Loeb *et al.* (1935) assembled the available data and concluded that the decrease of the concentration of sodium and chloride in the blood which occurs in Addison's disease and in adrenalectomized animals was because of loss of these electrolytes through renal excretion. Somewhat earlier Loeb *et al.* (1933) had concluded: "From the evidence presented it is difficult to ignore the possibility that this assumed influence of the adrenal glands may be exerted on renal function." Harrop *et al.* (1933a) expressed the belief that one function of the cortical extract in the adrenalectomized dog is that of participation in the regulation of the metabolism of sodium and chloride: "The available evidence points to the kidney as the locus of this regulatory function of the cortical hormone."

The changes which occur in different species of experimental animals are not the same. During adrenal insufficiency in man, the cat, and the dog, sodium and chloride are rapidly lost through the kidney

(Harrison and Darrow, 1938), but in the opossum this is not so (Britton and Silvette, 1937a; Hartman *et al.*, 1943). The concentration of sodium and chloride in the plasma may increase (Silvette and Britton, 1936). The response of the adrenalectomized opossum to the ingestion of a solution of sodium chloride and of the normal opossum to an extract of the adrenal cortex was less than that shown by other mammals (Silvette and Britton, 1938; Smith *et al.* 1943).

Sandberg *et al.* (1937) did not find an increase of the concentration of sodium in the urine of the adrenalectomized rat. The concentrating power of the kidney was impaired but in the adrenalectomized dog both the total amount of sodium and its concentration in the urine were increased.

2. *The Excretion of Potassium*

Harrison and Darrow (1939) found that after adrenalectomy, associated with the loss of sodium, the concentration of potassium in the urine was much lower than that found in normal animals with comparable concentrations of these ions in the plasma. The limiting ratio of the potassium in the urine to that in the plasma (the *U/P* ratio) was approximately 10:1 compared to 40:1 to 75:1 for the normal dog. Keith and Binger (1935) have shown that in man under normal conditions the concentration of potassium in the urine may be 50 times that in the plasma. Harrison and Darrow (1939) concluded that the abnormality of the excretion of potassium after adrenalectomy was not dependent on the change in the glomerular filtration, and failure to concentrate potassium in the urine was not because of inability of the kidney to do osmotic work nor to diuresis. The total excretion of electrolyte was not low, and even at the same volume of urine the administration of an extract of the adrenal cortex rapidly increased the *U/P* ratio for potassium. The disturbance of function involved the differential absorption of potassium by the tubules of the kidney.

3. *Glomerular and Tubular Activity*

In the untreated adrenalectomized dog it appears probable that both the rate of glomerular filtration and that function of the renal tubules which is concerned with the differential reabsorption of electrolytes are impaired (Gersh and Grollman, 1939). Harrison and Darrow (1939) suggested that as a result of the disturbed function of the tubules the concentrations of electrolytes in the body fluids are not maintained at normal levels. The decrease of electrolyte is followed by a shift of water from the extracellular to intracellular spaces and a decrease of the volume of the extracellular fluid. As a consequence of this the volume of the

plasma is decreased and in turn the blood pressure is diminished. In the adrenalectomized dog, and in the intact dog depleted of sodium and chloride, the decreased rate of glomerular filtration, which may be only 25% of the normal value, can be explained by the decrease of blood pressure. As the terminal stage of adrenal insufficiency approaches, the decrease of blood pressure would be still further aggravated by other factors such as a change of the muscular tone of the vascular system. Harrison and Darrow (1939) have postulated that the retention of urea in adrenal insufficiency can be adequately explained by the decreased rate of glomerular filtration together with an increased reabsorption of urea which is secondary to the reduced glomerular filtration.

These same investigators also pointed out that in the intact animal during the process of differential reabsorption of water and electrolytes in the renal tubules high concentration gradients between tubular urine and blood plasma may be developed. After adrenalectomy in the dog there is a marked disturbance of renal function of a specific type. Sodium is not adequately reabsorbed from the glomerular filtrate at a time when the concentration of sodium in the plasma is low. Potassium is reabsorbed to a much greater extent in the renal tubules of the adrenalectomized dog than in the normal animal at a time when the concentration of potassium in the plasma is abnormally high.

4. The Excretion of Water

Moderate diuresis frequently accompanies the increased excretion of sodium and chloride after adrenalectomy or after cessation of treatment of an adrenalectomized dog with cortical extract (Banting and Gairns, 1926; Corey, 1927; Loeb *et al.*, 1933; Swingle *et al.*, 1934c; Harrop, 1936b). However, the increased loss of water through the kidneys is but transitory and after a few days if water is administered diuresis does not follow (Gaunt, 1944a). The abnormality which develops in adrenal insufficiency may be influenced by four possible causes: (1) delayed absorption of water from the gastrointestinal tract; (2) an abnormal amount of the antidiuretic hormone in the blood; (3) an abnormal distribution of water in the body; (4) faulty excretion by the kidney (Gaunt, 1944b; Levy *et al.*, 1946).

1. The absorption of water from the gastrointestinal tract is delayed but this cannot be the only or the most important cause for the failure to produce diuresis.

2. Both the adrenal cortex and the antidiuretic hormone are concerned with water metabolism. Adrenalectomy in cats appears to be followed by the excretion of increased amounts of an antidiuretic substance (Martin *et al.*, 1939). The results of Joseph *et al.* (1944) on

adrenalectomized rats indicated almost complete repair of the delayed diuresis by small doses of cortical extract. Gaunt (1944a) concluded that the posterior lobe of the hypophysis can be eliminated by implication from the responsibility for the antidiuretic influence found in adrenalectomized rats, for hypophysectomized rats failed to show a normal diuretic response to water and Chen and Geiling (1943) reported that this occurred whether the posterior lobe was intact or removed. Since it was found that the failure to produce diuresis in the hypophysectomized rats was relieved by administration of cortical hormones it follows that the primary fault was adrenal insufficiency. By implication the posterior lobe of the hypophysis was not of first importance as a cause for failure to show a normal diuretic response to water after adrenalectomy.

3. Some degree of hydration of the intracellular compartment is usually a sequel to insufficiency of the hormones of the adrenal cortex. Although concentration of the circulating fluid is encountered only in severe insufficiency the tendency for hydration of the cells is apparent at an early stage. This situation would probably modify the availability of water for excretion by the kidney.

4. To explain the failure of the normal diuretic response to water as faulty excretion by the kidney, only little help, if any, is given by a histologic study of the kidney. The appearance of the structure is usually essentially normal (Levy *et al.*, 1946).

It is probable that all four causes which have been discussed play a part. Shipley (1945) concluded that although there is a demonstrable delay of the intestinal absorption of water in adrenalectomized rats and a decreased emptying time of the stomach, these are only minor causes of failure of excretion when water is ingested in large quantities. The greatest portion of the administered water is retained in the extracellular compartment and probably remains unexcreted because of failure of elimination by the kidney.

VII. THE TRANSFER OF WATER AND ELECTROLYTES THROUGH THE INTESTINAL WALL

Visscher and his associates have proposed a hypothesis for the transfer of water and certain electrolytes between the lumen of the intestine and the blood, which indicates a possible explanation for some of the observations recorded in the preceding pages. They pointed out that the ability of living systems to perform osmotic work is generally recognized but that the mechanism by which such processes occur has not been elucidated. The renal tubules are able to bring about the movement of chloride from the glomerular filtrate to the plasma until the concentration of chloride within the tubules is virtually zero. To move the last portions

of the chloride the renal cells transport ions from one concentration to another which is at least 100 times as great. Such a process as in the kidney is difficult of analysis, for it is necessary to deal with microscopic structures and very small amounts of material. It was shown that a comparable phenomenon could be made to occur in the small intestine of the dog and there was thus made available a convenient preparation for the study of the transportation of ions (Ingraham and Visscher, 1936a).

In summary, the mechanism suggested is a simultaneous forced flow of water across the intestinal epithelium in both directions. Differences of the electrolyte content of the water in the two streams and the relative rates of the streams determine the direction and magnitude of the net transport (Visscher *et al.* 1944b).

This conclusion was formed through the use of deuterium oxide and isotopic sodium and chloride. The experimental results were found to differ as much as 200-fold from the values which would be obtained if the movement of water, sodium, and chloride was by diffusion. In certain instances the direction of movement was the reverse of the predicted. The observations refute the commonly held belief that net water movement between the ileum and the blood occurs primarily because of normal osmosis. They are also incompatible with the view that differences of concentration in the normal intestine and in the blood are the major causes for the movement of sodium and chloride ion between the two fluids.

The apparent concentrations of the sodium and chloride ions in the water which passed from the lumen of the intestine into the blood were proportional but not always equal to the concentrations of these ions in the intestine, but the apparent concentrations of sodium and chloride in the water which moved from the blood into the intestine were practically independent of the concentrations of these ions in the intestine. The turnover of sodium between the intestine and the blood appears to be rapid and the results indicate that an amount of sodium approximately equal to the total sodium in the plasma may be replaced in less than 100 minutes (Visscher *et al.* 1944a).

When distilled water or hypotonic solutions of sodium chloride or of urea were placed in the small bowel of a dog the concentrations of sodium, chloride, and urea increased to values which were greater than their respective concentrations in the blood. However, in the presence of the sulfate ion, chloride did not accumulate but on the contrary was removed from the bowel until its concentration was much less than that of the blood plasma.

The sulfocyanate ion in the bowel affected the movement of chloride through the intestinal wall in the opposite way from that of sulfate and

caused the chloride ion to accumulate to a concentration which was greater than that in the blood plasma (Burns and Visscher, 1935).

The poisons, arsenite, fluoride, sulfide, cyanide, and mercuric chloride, abolished the action of sodium sulfate and in the presence of these poisons chloride moved into the intestine under circumstances such that it would otherwise move out. In addition, in the presence of these poisons the intestinal wall, which normally was impermeable to sulfate, became permeable to it (Ingraham and Visscher, 1936b).

The results of Peters (1941) supported the experimental work on the circuit hypothesis and Driver (1942) also found that in the presence of sulfate ion, chloride rapidly passed from the lumen of the intestine. Hexylresorcinol and other agents were shown to modify the transport of electrolytes through the intestinal wall.

At the present time investigations to clarify the factors which influence the transfer of electrolytes have been limited, but Dennis and Wood (1940) have shown that after adrenalectomy there are changes in the absorption of ions from the intestine similar to those observed by Harrison and Darrow (1939) in the kidney. There was a tendency for the absorption of potassium from the lumen of the intestine and for loss of sodium from the blood into the intestine. Whether these changes were brought about by a specific effect of the state of adrenal insufficiency has not been shown; however, administration of an extract of the adrenal cortex corrected this abnormal behavior.

VIII. THE RELATION BETWEEN SODIUM SALTS AND THE HORMONES OF THE ADRENAL CORTEX

Perhaps the most significant single fact which has resulted from many years of investigation of adrenal insufficiency is the specific nature of the effect of sodium chloride. Completely adrenalectomized dogs which have been maintained for months with the optimal amounts of sodium, potassium, and chloride are alert, vigorous, and strong and to all outward appearance they cannot be distinguished from normal dogs. Furthermore, the mineral and organic constituents of the blood and tissues are normal and histologic examination of the muscles, the gastrointestinal tract, the kidneys, the glands of internal secretion, and other organs reveals the fact that except for enlargement of the thymus and the lymph nodes these structures are essentially normal.

These results would indicate that loss of sodium chloride through the kidney is a principal cause for the severe prostration, asthenia, and death after adrenalectomy and that restoration of a normal condition is dependent on replacement of the lost electrolytes. As a general description of the course of events this statement is correct, but when a detailed

study is made it becomes clear that the relationship between many physiologic processes and the intake of sodium chloride is complex. The effects are so broad in scope that it is impossible to select any primary locus. The following paragraphs record some of the relationships that have been observed.

IX. THE INFLUENCE OF SODIUM CHLORIDE ON PHYSIOLOGIC PROCESSES

1. Renal Functions

Water. The influence of sodium chloride on renal function was shown by the response of adrenalectomized dogs to intravenous injection of isotonic solutions of glucose. If 0.9% sodium chloride was present in the solution adrenalectomized dogs responded like normal dogs. Diuresis was induced but the glucose was not injected at a rate fast enough to cause glycosuria, and there were only minor changes in the electrolytes of the blood after the solution had been injected for 3 hours. It was also found that an equally satisfactory result was obtained if the sodium chloride was omitted, but an extract of the adrenal cortex was given. However, if the isotonic solution of glucose alone was injected the dogs became anuric and severe symptoms of adrenal insufficiency developed (Kendall *et al.*, 1938). Gaunt (1944a) found that the injection of only 1 cc./hour of physiologic salt solution into normal rats while large doses of water were being given by mouth increased diuresis, extended life, and prevented the rise in the percentage of cells in the blood as measured by the hematocrit.

Sodium and Potassium. Anderson (1943) suggested that the administration of sodium chloride not only replenished the body with the needed ions but also restored to the kidney the "mechanism which is responsible" for the normal excretion of sodium and potassium. To demonstrate this, radioactive sodium and potassium were given to rats at various times after adrenalectomy and it was shown that after 8 days an average of 60% of the ingested radioactive sodium was excreted during a 48-hour interval when the rats drank tap water. The average amount excreted by normal rats was 37% and the value for adrenalectomized rats maintained with 1% solution of sodium chloride was 36%. For potassium the value for the normal animal was 10%. Adrenalectomized rats given tap water for 8 days excreted only 5%; however, when adrenalectomized rats were given 1% solution of sodium chloride for intervals of 5 and 8 days, 10 and 11% respectively of the radioactive potassium was in the urine.

At the present time it is difficult to interpret this apparent return of normal renal function in any greater detail than Anderson's suggestion

that the mechanism responsible for the normal excretion of sodium and potassium had been restored by the ingestion of sodium chloride.

Kottke *et al.* (1942) found that after the adrenalectomized dog had been maintained in good condition for an interval on a diet with high sodium and low potassium content the capacity of the kidney to excrete a dilute urine was only slightly, if at all, impaired when compared with the capacity of the kidney of the intact animal. The kidney of the adrenalectomized dog was not able to produce as concentrated a urine nor one with as high a content of sodium chloride as the kidney of an intact dog. However, to show these slight limitations it was necessary to resort to severe measures during which the temperature of the environment was elevated.

These results, which are in keeping with those of Anderson, show how nearly normal the kidney had been maintained without the hormones of the adrenal cortex by the use of a diet which had an optimal concentration of electrolytes.

Excretion of Urea. Banting and Gairns (1926) appear to have been among the first investigators to demonstrate the close relation between the excretion of urea and the administration of sodium chloride in the adrenalectomized dog. They reported that in the morning of a certain day the concentration of urea of an adrenalectomized dog was 315 mg./100 cc. blood. In the afternoon, despite the fact that the dog had received 300 cc. of serum, the concentration of urea was 353 mg. Two hundred cubic centimeters of 5% saline solution were given and on the following morning the blood urea had decreased to 109 mg. Two hundred and seventy-five cubic centimeters of 5% saline solution given during the day reduced the concentration of urea to 90 mg. in the afternoon and the following morning the value was 60 mg.

This work had been preceded by that of Marshall and Davis (1916) who showed a marked rise in the blood urea after adrenalectomy, and it was followed by the observations in many laboratories that an increase of the nonprotein nitrogen and urea in the blood are invariable sequelae to adrenalectomy.

Harrison and Darrow (1939) have pointed out that there are adequate reasons for the increase of urea, which are based on the abnormal distribution of water in the intracellular phase, the extracellular phase and the volume of the blood; and these investigators showed that a decrease of the content of sodium chloride in the blood, produced by an intraperitoneal injection of glucose, will result in an almost complete suppression of the excretion of urea.

Swingle and Remington (1944) suggested several reasons for a decrease of the clearance of urea, among which is a decrease of blood pressure. Swingle *et al.* (1934b) observed that the increase of the con-

centration of urea in the blood occurred simultaneously with the fall of the arterial pressure and did not precede it. It was believed that failure to excrete urea was probably extrarenal in origin and was not necessarily related to the kidney. However, the phenomenon appeared to be related to the change of the concentrations of the electrolytes in the blood.

Adrenalectomized dogs, when given small sustaining amounts of cortical extract and fed ground meat which had been freed of potassium by boiling in water, may be strong, vigorous, and with good appetite with a concentration of urea of from 100 to 400 mg./100 cc. blood. These experiments were not acute nor of short duration. The concentration of urea could be held at almost any desired value by variations of the daily intake of sodium chloride (Kendall, unpublished results). These results may be explained quite simply on the grounds already suggested (that is, a decrease of blood pressure or a decrease of the extracellular compartment), but it is possible that something more specific for renal function is supplied by sodium chloride. As with the excretion of water and of sodium and potassium ions, sodium chloride may in part "restore the mechanism responsible" for the excretion of urea by the kidney.

2. *Intestinal Absorption*

Electrolytes. Dennis and Wood (1940) found that administration of sodium chloride to an adrenalectomized dog restored the function of the intestine to such a degree that the constituents of the blood were essentially normal and the dog was objectively in excellent health; however, the behavior of the intestine was not normal. The rate of absorption of sodium in general declined more than that of potassium and there was often a reversal in direction of the net movement of sodium, which was sometimes excreted into the lumen of the intestine although potassium was still being absorbed.

Clark (1939) and Stein and Wertheimer (1941), with adrenalectomized rats maintained with sodium chloride, fully confirmed the results of Dennis and Wood. It was also shown that resorption of sodium chloride from a low concentration in the intestine when the concentration in the blood was higher was no longer possible.

Glucose. Verzár (1939) contended that there is a failure of the selective absorption of metabolizable sugars after adrenalectomy. Clark and MacKay (1942) have adequately explained this observation. They found that the rate of absorption of glucose was markedly reduced when measured immediately (24 hours) after the removal of the adrenal glands. When adrenalectomized rats were given sodium salts and maintained for 2 weeks after operation but were not allowed salt water during a period of 36 hours before testing, or if only tap water was given throughout the

period of observation, the absorption of glucose was significantly lower than in the controls. When administration of sodium chloride was continued until the observations on the absorption of glucose were made, the rate of absorption in the adrenalectomized rats was the same as that in the controls. It was concluded that the impaired absorption of glucose evident in adrenalectomized animals under some conditions was not referable to the lack of the influence of the hormones of the adrenal cortex on the intestinal mucosa, but rather to the consequent disturbance of salt metabolism. Similar results have been reported by Anderson (1943) and by other investigators cited in the work of Clark and MacKay (1942).

Fat. Iaszt and Verzár (1936) found that after adrenalectomy in the rat absorption of fat was decreased. This result was interpreted as a failure of phosphorylation of the fat in the absence of the adrenal cortex, but Barnes *et al.* (1941a) reported that when the adrenalectomized rats were maintained in good condition with sodium chloride the absorption of fat was normal. Subsequently this conclusion was modified (Bavetta *et al.*, 1941; Bavetta, 1943; Bavetta and Deuel, 1942) to the extent that the lower fatty acids up to caproic are absorbed at a normal rate by the adrenalectomized rat maintained with sodium salts. However, under the same experimental conditions the higher fatty acids are not removed as rapidly from the intestine of the adrenalectomized rat as from the intestine of the normal rat. In adrenalectomized animals maintained on water alone, Bavetta and Deuel (1942) showed that the absorption of sodium butyrate was significantly lowered but the rate was restored to normal by administration of sodium chloride. It was suggested that the beneficial effect of sodium salts was through dilution of the blood and restoration of a more normal circulation. When excretion of fat and fatty acids in the feces was used as a criterion it was found that adrenalectomy did not influence the absorption of fat in rats, provided the intake of food was adequate and the rats were maintained with sodium chloride in the drinking water (Clark and Wick, 1939).

3. Metabolism of Protein

Anderson (1943) and her associates have investigated the influence of adrenalectomy on growth, consumption of food, and the need for the optimal intake of sodium chloride. Without the addition of sodium chloride growth stopped, and the food and water intake decreased. With a daily intake of 700–900 mg. sodium chloride the rats survived indefinitely and the rate of growth was almost the same as that of normal controls. An intake of about 350 mg. was insufficient for growth or survival and when the intake was approximately 1,200 mg. the rats

resembled those that were untreated; the survival time was less than 20 days.

The adrenalectomized rat with optimal amounts of sodium chloride is apparently able to utilize exogenous protein almost as well as is the normal rat. After treatment with phlorhizin, the adrenalectomized rat is fully able to convert exogenous protein into glucose (Wells and Kendall, 1940b), but Evans (1936), Long *et al.* (1940) and Anderson (1943) have shown that the adrenalectomized rat cannot readily utilize endogenous protein during a fast, and a limited ability of the adrenalectomized rat after treatment with phlorhizin to convert endogenous protein into glucose was found by Wells and Kendall (1940b).

Under fasting conditions, when the optimal intake of sodium chloride was given, not only were adrenalectomized rats able to survive longer than when the intake of sodium chloride was not optimal but the excretion of more nitrogen indicated that the rats were able to utilize endogenous protein to a greater extent. With too little or too great an intake of sodium chloride the utilization of protein was decreased (Anderson, 1943).

More recently Ingle and Oberle (1946) have completed work which increases the significance of sodium chloride in relation to the metabolism of endogenous protein. Rats were first adapted for a period of 2 weeks to a regimen of forced feeding and were then adrenalectomized. The adrenalectomized rats were given a 1% solution of sodium chloride to drink during all phases of the experiment. During the first postoperative day the adrenalectomized rats excreted less nitrogen than before adrenalectomy, but by the third day there was an increase of the excretion of nitrogen. The higher rate of excretion was sustained for several days and was greater in the adrenalectomized rats than in the control intact rats. During a 10-day fast the adrenalectomized rats excreted as much nitrogen as did the control animals.

These results, and also those of Anderson, furnish positive evidence for the ability of an adrenalectomized rat to utilize endogenous protein. Groat (1941) reported that fasted adrenalectomized ground squirrels given 1% solution of sodium chloride to drink did not survive any longer than did a similar group given tap water. Groat also reported that adrenalectomized rats given 1% solution of sodium chloride to drink did not survive longer than adrenalectomized rats given tap water when the rats were fasted or given a limited amount of food.

It is possible that these results may be explained by failure of the adrenalectomized animals to become adapted to the conditions imposed by the fast, but whatever explanation is given the result is negative in nature and is not confirmed by subsequent work. This is mentioned

because Darrow (1944) referred to the work of Groat and drew the conclusion that "these experiments give further evidence that adrenal insufficiency is not simply a matter of water and electrolyte balance."

The work of Ingle and Oberle (1946) indicates that in rats that have been adapted to the experimental conditions, as long as there is a balance of water and electrolytes, those processes concerned with utilization of endogenous protein proceed as well in the adrenalectomized rat as in the normal rat. Moreover, during a fast a severe strain is imposed on the animal, not only in regard to the utilization of endogenous protein but in the maintenance of homeostasis under adverse conditions. Even this test has now been passed satisfactorily by the adrenalectomized rat when maintained with the optimal intake of electrolytes.

4. Metabolism of Carbohydrate

Deposition of Glycogen in the Liver. Long *et al.* (1940) have reviewed the influence of the adrenal cortex on carbohydrate metabolism. It has been a subject of much investigation and it has also been a source of controversy since 1930, when Britton and his associates attempted to relate the function of the adrenal cortex to the observation that in the adrenalectomized animal under certain conditions only small amounts of glycogen were present in the liver and muscles, and that hypoglycemia was an almost constant sequel to adrenal insufficiency. Throughout the following decade the dependence of carbohydrate metabolism on the hormones of the adrenal cortex and the secondary importance of "the water and salt hormone" were emphasized by Britton and his associates (Britton and Silvette, 1931, 1932a, 1932b, 1934, 1937b, 1937c; Britton, 1932; Silvette and Britton, 1932, 1936; Britton *et al.*, 1938).

While the close link between the hormones of the adrenal cortex and carbohydrate metabolism was being emphasized by Britton and his associates, results in other laboratories tended to minimize this phase of adrenal insufficiency. The conclusion was drawn that after adrenalectomy carbohydrate metabolism was not primarily disturbed (Parkins *et al.*, 1936). Deuel *et al.* (1937) found that the deposition of glycogen was not altered by adrenal insufficiency if the rats were maintained in good condition by oral administration of sodium chloride. However, two years later Britton (1939) reported that the adrenalectomized cat maintained with solutions of glucose and sodium chloride by mouth was unable to fix or synthesize glycogen in the liver.

Long *et al.* (1940) bridged the gap with a probable explanation for the divergence in experimental results. They pointed out that "the conditions under which a solution of this problem may be attempted require the recognition of certain complicating factors that are encountered in

dealing with adrenalectomized animals. The first of these is the question of the food ingested prior to the glycogen analyses. In some species, particularly the cat, all food may be refused for several days before death, while in others food may be eaten up to a few hours before it occurs. Obviously, this difference will greatly influence the levels of carbohydrate in the tissues when the examination is made. Arising from this is a second problem. Britton and his colleagues have recently reaffirmed that not only do adrenalectomized animals of several species exhibit reduced carbohydrate levels, but they also show a much decreased ability to form liver and muscle glycogen from ingested carbohydrate." Long *et al.* (1940), in agreement with Deuel *et al.* (1937), showed that adrenalectomized rats or mice, maintained in good health by the oral or intraperitoneal administration of sodium salts and fed glucose, showed no marked abnormalities in the storage of carbohydrate. However, when fasted, there was a rapid decline of the hepatic glycogen and an abnormal fall of muscle glycogen which became progressively greater as the fast was continued.

Anderson (1943) and her associates added the important point that the maximal deposition of glycogen in the liver occurred only with the optimal intake of sodium chloride. The deposition of glycogen after absorption of fed glucose in adrenalectomized rats was close to the amount found in normal rats when both groups were given the optimal amount of salt. This was 1% of sodium chloride in the drinking water; however, if the intake of salt was too low or was more than optimal, then less glycogen was deposited in the liver. Furthermore, the beneficial effect of sodium chloride on the deposition of glycogen was not temporary in its action. The capacity of the adrenalectomized rat in this respect persisted for several months if the rat was maintained with 1% sodium chloride in the drinking water.

At the end of the decade it was shown that the secretion of the adrenal cortex contains not one but a group of hormones. Some exert an effect on the storage of carbohydrate and these have but a limited influence on the metabolism of electrolytes. Others have a specific effect on the excretion and distribution of electrolytes, but these do not modify in a significant way the storage of carbohydrate. Long *et al.* (1940) furnished convincing evidence for the effect of corticosterone, dehydrocorticosterone, and the 17-hydroxyl derivatives of these two hormones on the deposition of glycogen in the liver.

Gluconeogenesis. In addition to the deposition of glycogen in the liver of adrenalectomized animals after the ingestion of foodstuff, it was reported by Britton and Silvette (1932a, 1932b) that the injection of an extract of the adrenal cortex increased the blood sugar and the glycogen

in the muscle. These observations were confirmed by Thaddea (1935) but Parkins *et al.* (1936) failed to note a significant effect of the extract of the adrenal cortex on blood sugar. At the time when these experiments were performed there was always a question whether traces of epinephrine or other toxic compounds were present in the extracts of the adrenal cortex that were used, so that it was difficult to evaluate the results.

When corticosterone and its derivatives became available it was possible to study the effects of these pure crystalline hormones on carbohydrate metabolism. This study was carried out by Long and his associates in a convincing manner (Long *et al.*, 1940). They were able to show that certain crystalline hormones of the adrenal cortex increased the level of glucose in the blood of both normal and adrenalectomized rats and mice. In addition the total store of carbohydrate in the body was increased and this occurred even when the animals were fasted before the hormones were administered.

This effect on carbohydrate metabolism was apparently confined to corticosterone, dehydrocorticosterone, and the two derivatives of these compounds with a hydroxyl group at C₁₇.

The increase of the concentration of blood sugar confirmed the results obtained by Britton and his associates but the rise in the glycogen of the muscle which had been reported by Britton was not confirmed. The increase which was found by Britton was ascribed by Long *et al.* (1940) to the manner in which the experiment was performed.

When it was evident that the total carbohydrate stores in the body were increased by treatment with the hormones of the adrenal cortex it became a matter of importance to explain the source of the newly formed sugar. It was suggested by Long *et al.* (1940) that, since there was an increase of the excretion of nitrogen, and in the absence of experimental evidence for the transformation of fat to carbohydrate, the most probable source seemed to be protein. The increase of the excretion of nitrogen indicated the utilization of an amount of protein which could account for the gluconeogenesis.

In the experiments that have been described the adrenalectomized rats were maintained in good health with a high daily intake of sodium chloride. In this condition, even during a fast, the conversion of protein to glucose proceeds at a rate sufficient to sustain the concentration of glucose above the level at which symptoms of hypoglycemia appear; however, through the use of sodium chloride alone the rate of utilization of endogenous protein cannot be increased to the point at which glycogen is deposited in the liver. Evans (1936), and Wells and Kendall (1940b) have shown that even the stimulus to protein catabolism which is asso-

ciated with phlorhization does not result in a high excretion of glucose by the adrenalectomized rat when maintained on sodium chloride alone. The administration of corticosterone and related hormones increased the glycosuria to that observed in the normal rat. The source of the glucose in this case was apparently protein since the D:N ratio was 3.7:1.

5. *Metabolism of Fat*

Verzár and Laszt (1936) noted that the fatty livers normally found in rats that have been poisoned with phosphorus did not develop if the animals were adrenalectomized. In such animals administration of an extract of the adrenal cortex was followed by deposition of fat in the liver. MacKay and Barnes (1937) and MacKay (1937) investigated the deposition of fat in the liver in a wide range of conditions such as during fasting, after the existence of a state of alkalosis, or if the animal was given heavy cream or a high fat-low protein diet.

Since the deposition of fat in the liver under these conditions did not occur in the adrenalectomized rat although under the same conditions fat was deposited in the liver of the intact animal, MacKay and associates became interested in the influence of adrenalectomy on fat which had previously been stored in the liver. It was found that in the normal animal the excess fat gradually left the liver over a period of several days, but if the rats were adrenalectomized when the fast was begun the fat stored in the liver was removed from this organ much more rapidly.

The prevention of fatty livers in adrenalectomized rats was observed even though they were maintained in good condition with sodium chloride, but if the rats were given an active extract of the adrenal cortex they behaved as normal rats.

Barnes *et al.* (1941b) confirmed the observation that fat was not deposited in the liver in adrenalectomized rats maintained with sodium chloride. Fatty acids with a strong absorption of radiation in the ultra-violet region through the effect of conjugated double bonds were used to follow the rate of phosphorylation and deposition in the liver. The results indicated that in the adrenalectomized rat maintained with salt the tagged fatty acids were not deposited in the neutral fat of the liver within 8 hours after the fat was fed, but when cortical extract was given, the tagged fatty acids were deposited as neutral fat in the liver.

Observations of the incorporation of tagged fatty acids into the phospholipid fraction of the liver indicated that in the adrenalectomized rats maintained only with sodium chloride there was a normal or possibly an increased rate for the phosphorylation of the fatty acid. Treatment with cortical extract did not modify this phase of the metabolism of fat.

6. *Metabolism of Potassium*

There are strong indications that one of the most important reasons for the favorable effect of sodium chloride in adrenal insufficiency is the specific influence exerted by this salt on the distribution and excretion of potassium.

When adrenalectomized dogs were maintained with a high intake of sodium chloride and sodium bicarbonate an inverse relationship was found between the concentration of sodium and potassium in the serum (Harrop, 1936a). If the concentration of sodium was below the normal value because of an inadequate intake then the concentration of potassium increased, but if the intake of sodium salts was sufficient the abnormally high level of potassium decreased, often to the normal value. This decrease of potassium in the serum was associated with an increase of its urinary excretion, frequently with a well-marked diuresis.

Nilson (1937) showed that the asthenia and prostration which are induced by the ingestion of potassium salts by an adrenalectomized dog can be relieved by the administration of sodium chloride and sodium citrate alone. If the condition of the animal was not critical it was not necessary to resort to the use of an extract of the adrenal cortex to restore the normal condition. The beneficial effect of sodium salts in Nilson's experiment was closely associated with the decrease in the concentration of potassium in the serum.

Harrison and Darrow (1938) stated that "the mechanism by which changes in the concentration of sodium in extracellular fluid may influence the concentration of potassium within the muscle cell is unknown." Buell and Turner (1941) expressed a similar thought: "The mode of action of sodium chloride in compensating for the specific effects of adrenal insufficiency in the muscles is not clear. There is no evidence here of increased permeability of cell membranes; in fact, there appears to be less sodium rather than more inside the muscle cells. The fact remains, however, that treatment with sodium chloride has in some way prevented the increase in muscle potassium which would have occurred in its absence."

The demonstration that there is not only an inverse relation between the intake of sodium chloride and the excretion of potassium but that an inverse relation also applies to the concentrations of sodium and potassium in the plasma, together with the observations of Miller and Darrow that the concentration of potassium in the muscle varies with that in the plasma, provides the necessary conditions for the specific effects of sodium chloride. However, the details of the mechanism which is the basis for the inverse relationship between the electrolytes have not been revealed.

It has been shown by Heppel (1939) that the influence of sodium chloride on the metabolism of potassium reaches still further. Even in normal rats a large fraction of the potassium within the muscle cells can be replaced with sodium. This was accomplished by use of a diet in which the content of sodium chloride was high but that of potassium was low.

Following the work of Heppel (1939) on replacement of potassium in muscle with sodium *in vivo*, Steinbach (1940) showed that as potassium was soaked out of frog muscle by treatment with potassium-free Ringer's solution, sodium entered in exchange. The exchange was reversible as long as the muscles did not lose more than about half of the original potassium, and provided that the chloride content of the tissue remained about the same.

7. Miscellaneous Effects

Appetite. A clear demonstration of the general systemic effect of sodium chloride on adrenalectomized rats is furnished by the procedure devised by Groat (1941). Adrenalectomized rats were maintained with 1% solution of sodium chloride. The salt solution was then replaced with water. When it appeared that the rat would succumb from adrenal insufficiency the water was replaced with 1% solution of sodium chloride. Substitution of water for the salt solution was followed by a loss of weight. After three days the average body weight was approximately 90% of the starting weight and the average intake of food was only 45% of the former intake. Even the most extreme of 3-day fluctuations in the food intake of normal rats as found by Groat were small in comparison to these figures. After replacement of the water with 1% solution of sodium chloride it was observed that the rats immediately began to drink. A large quantity of the solution was consumed within an hour or two and the rats then exhibited a normal appetite. In a group of six animals the average gain in weight after 1 hour was about 25 g., most of which was water.

Some interesting observations (Richter, 1936b, 1941; Richter and Eckert, 1938; Clark and Clausen, 1943) indicated the apparent wisdom of adrenalectomized rats when given a choice of food and water with and without added sodium chloride. The rats voluntarily selected a diet which afforded a high daily intake of salt. The probable basis for this selection was an increase in the sensitivity of the sense of taste to salt (Richter, 1939).

Spontaneous Activity and Response to Stimulation of Muscle. Richter (1936a) observed that the spontaneous running activity of rats rapidly decreased to zero after adrenalectomy and that when small amounts of sodium chloride were added to the diet the activity increased. When the

content of sodium chloride in the diet was made 5% the activity of the rats was raised to a level practically as high as in adrenalectomized rats that were treated with an extract of the adrenal cortex.

Adrenalectomized rats that were maintained on a diet high in sodium chloride and low in potassium were able to perform more work, as measured by the method of Ingle, than other adrenalectomized rats given a diet of dog chow. However, the work of all adrenalectomized rats was only a small fraction of that of a normal rat (Ingle, 1940).

Toxicity of Certain Drugs and Salts. Laszt (1939) by treatment with sodium chloride was able to counteract the toxic effect and to restore the impaired intestinal absorption of glucose which follows the administration of iodoacetic acid to normal animals. Clark and Barnes (1940) confirmed this result in a striking manner. When a group of normal rats were given sodium chloride-sodium citrate in the drinking water all survived the injection of 80 mg. of iodoacetic acid but another group given water alone lived on the average only 8 hours after injection of 80 mg. of the same compound. A beneficial effect of sodium chloride was also shown when colchicine and mercuric chloride were given to normal rats compared with other rats that were given water alone or cortical extract.

Rapoport and Guest (1942) treated adrenalectomized rats with potassium and sodium phosphate and found that the lethal dose of the phosphate ion was about the same as that of potassium. However, when sodium chloride was given, the tolerance of all of the adrenalectomized rats was much increased even when the phosphate was given with a small amount of potassium ion.

Amberg and Helmholz (1919) were able to protect guinea pigs from the toxic effect of potassium chloride by the simultaneous injection of sodium chloride. The time relationships excluded the kidney from consideration as a factor and if the sodium chloride was given first in a separate solution the protection afforded against the subsequent injection of potassium chloride decreased with time. Sodium acetate did not protect the animals from the toxic effect of potassium chloride. Amberg and Helmholz described the result as an example of the antagonistic effect of sodium chloride to potassium and it is difficult to explain the experiments in any other manner.

Intestinal Response to Vasoconstrictor Influences. Fowler and Cleg-horn (1942) observed that a noteworthy feature of most cats dying of adrenal insufficiency was the highly contracted state of the small intestine. When such an animal was given physiologic solution of sodium chloride by intravenous injection the response of the intestine to stimulation of the splanchnic nerve or to epinephrine was restored to normal. This was

interpreted as showing that the original contracted state was caused by a decrease of the blood volume or by the ionic ratio of sodium, potassium, and chloride in the intestine.

Metabolism of Melanin. Spoor and Ralli (1944) investigated the hides of rats in regard to the formation of melanin. The rats were given various diets and some were adrenalectomized. On a diet deficient in the filtrate factors of vitamin B there was a decrease of the extractable melanin of the skin. Dehydration by means of 2% solution of sodium chloride or by deprivation of water produced chemical changes in the skin which simulated to a certain extent those induced by a deficiency of the filtrate factor. Adrenalectomy in rats fed the deficient diet resulted in an increase of the extractable melanin. The skins of adrenalectomized rats deprived of salt but fed diets supplemented with the filtrate factors did not contain greater amounts of melanin than the skins of normal rats. The results indicated that the metabolism of melanin was influenced by both the adrenal cortex and sodium chloride.

Changes in the Anterior Pituitary Body. Koneff *et al.* (1941) summarized their investigation of the effect of sodium chloride on the anterior pituitary body of the adrenalectomized rat as follows: "If we can draw any parallel between the morphological state of a cell and its capacity to perform its specific functions, then the administration of sodium chloride to the adrenalectomized rat results to a considerable extent in the preservation of the physiological status of the anterior pituitary chromophils.

"In view of our present knowledge of pituitary physiology it seems reasonable to assume that there is little or no deficiency in the secretion of thyrotropic hormone in the salt-maintained animal. The changes in the unsupported animal, on the other hand, make adequate secretion of this hormone impossible, the inadequacy of secretion being responsible for the hypoactive thyroid seen in adrenal insufficiency."

The Estrous Cycle and Reproduction. Kutz *et al.* (1934) found that all of a group of adrenalectomized rats not given sodium chloride (Rubin-Krick solution) remained in diestrous state until death, but those given a high intake of sodium chloride maintained a normal estrous cycle. This was interpreted as a result of the general ill health of the first-mentioned group rather than because of the absence of any specific hormone.

It was also shown that after complete adrenalectomy both male and female dogs remained fertile if they were maintained with sodium chloride and sodium citrate (Kendall *et al.*, 1936).

Erythropoiesis. Crafts (1941) observed a decrease of erythrocytes in rats for an interval of 10 to 20 days after removal of the adrenal glands when the rats were maintained with 1% solution of sodium chloride.

At the end of this period the erythrocyte count and hemoglobin returned to approximately normal values.

Basal Metabolism and Uptake of Oxygen. Brownell and Hartman (1941) found normal basal metabolic rates for adrenalectomized dogs that were maintained with sodium salts.

An intimate relation between consumption of oxygen and the concentration of sodium chloride was suggested by Cohn and Soskin (1943). The consumption of oxygen by dogs after depletion of sodium chloride by an intraperitoneal injection of glucose was abnormally low and was restored to the normal value by administration of a solution of sodium chloride sufficient to increase the volume of the blood to normal. The results indicated that the favorable effect of sodium chloride was exerted in large measure by the chloride ion, and the suggestion was made that this ion is involved in the mechanism for the transport and transfer of oxygen from blood to tissue. This work was continued with the study of the uptake of oxygen by rat muscle (diaphragm) (Cohn *et al.*, 1943). It was concluded that sodium chloride influenced the rate of dissociation of oxygen and hemoglobin and thus affected the amount of available oxygen.

X. THE SYMPTOMS OF ADRENAL INSUFFICIENCY

The investigations of Loeb and Harrop and their associates in 1932 to 1934 definitely related the symptoms associated with removal of the adrenal cortex with the metabolism of sodium, potassium, and chloride and with the abnormal distribution of water. During the following ten years many hypotheses were suggested to explain the cause of the symptoms and from these interpretations several issues arose which were controversial. It is not my purpose to dwell on the merits of the viewpoints, but a brief review of some of the experimental results may help to clarify the situation as it exists even today.

1. Sodium, Chloride and Water

All investigators have agreed that the loss of sodium and chloride is always associated with adrenal insufficiency, but Britton *et al.* (1938) pointed out that sodium and chloride could be decreased in the blood serum of normal animals to a value much below the concentration found after adrenalectomy and yet such animals did not show the symptoms of adrenal insufficiency. Therefore it was argued that the loss of sodium and chloride was of secondary importance. Harrop (1936b) mentioned work from six different laboratories which indicated that the decrease of blood volume in adrenal insufficiency was because "the blood water

slowly transudes into the tissues and interstitial spaces and is immobilized." The loss of water was believed to depend on the "inability to maintain capillary tone and normal permeability."

A second view proposed by Darrow and Yannet (1935) assumed the withdrawal of fluid from both plasma and interstitial spaces actually into the tissue cells because of the changes of osmotic pressure which followed the loss of sodium chloride through the kidney.

Investigations which have been carried out during the past twelve years have served to strengthen this hypothesis. For man, the cat, and the dog the symptoms of adrenal insufficiency follow the loss of sodium chloride which appears to be the primary departure from normal.

The abnormal distribution both of water and of electrolytes is satisfactorily accounted for by this hypothesis, and the dramatic result of treatment with sodium chloride would seem to complete the evidence and establish the validity of the original concept of Darrow and Yannet. Further work, however, has indicated the necessity of adding another chapter which contains many paradoxes and contradictions, but the accumulated investigations of the past ten years afford convincing evidence that intimately associated with the loss of sodium chloride as a primary cause of the symptoms of adrenal insufficiency there is another departure from normal; namely, retention of potassium.

2. Potassium

The presence of potassium in the intracellular compartment in a concentration approximately 20 times that in the serum; the maintenance of the concentration of this cation within a narrow range both in the cells and in the extracellular phase; the necessity for potassium for growth; the rapid changes in the concentration of potassium in the plasma associated with metabolic processes, with the administration of certain hormones and stimulation of muscle; the effect of the concentration of potassium on the contraction of muscle as shown by the prostration resulting from familial periodic paralysis and by the beneficial effects of an infusion of salts of potassium; the influence of potassium on certain enzymes as shown by *in vitro* studies; its essential function in the propagation of the nerve impulse; and the striking evidence of the toxicity of potassium after intravenous injection—all of these aspects of the physiologic importance of potassium suggest that a disturbance of the metabolism of this cation would cause deep-seated changes that would be associated with definite signs and symptoms.

The brilliant results obtained both with patients who had Addison's disease and with adrenalectomized dogs, when given only sodium salts, tended to obscure the fact that some of the symptoms of adrenal insuffi-

ciency were directly related to potassium. The best evidence for this relationship is the administration of small amounts of potassium salts to patients who have Addison's disease or to adrenalectomized animals that have been maintained with sodium chloride and sodium citrate. The results are striking, but before these are described other relations between potassium and certain physiologic processes and the toxicity of potassium will be discussed.

Muscle. To gain quantitative evidence for the relation between the concentration of potassium in the plasma and its effect on physiologic processes, investigations have been carried out on normal animals and the information thus obtained has been used to explain the behavior of animals after adrenalectomy.

The relation between potassium and muscular activity has been studied by Miller and Darrow (1940b, 1941) who concluded that within wide limits the amount of potassium in the muscle cells of normal rats does not limit the capacity to swim continuously for 60 minutes. Concentrations of potassium in the serum that are abnormally low are not in themselves sufficient to produce "paralysis" or muscular weakness in normal animals.

Darrow (1944) made the point that "most workers on adrenal insufficiency agree that the rise in muscle potassium is not the explanation of the muscular weakness but a more or less physiological reaction to the rise in concentration of serum potassium. Indeed, the reviewer has unpublished data on rats with chronic adrenal insufficiency which show low muscle potassium while on a diet low in potassium. The evidence seems clear, therefore, that the rise in muscle potassium is not a direct effect of lack of the hormone on the muscle but an indirect result of renal failure leading to retention of potassium and depletion of extracellular electrolyte."

Heart. In a study of the electrocardiographic changes of the heart Winkler *et al.* (1938) found in the normal dog the successive concentrations of potassium in the serum necessary to produce alterations in the T wave, depression of the S-T segment, intraventricular block, disappearance of the P wave and arrest of the heart, and Winkler *et al.* (1941) determined the same relations in adrenalectomized dogs. The concentrations of potassium necessary to produce these effects were approximately the same in each series but the amount of potassium which was necessary to raise the content of this cation in the serum of the adrenalectomized dogs was only a third that required for the intact dog. The rate at which potassium was administered was found to be an important factor. Potassium was more toxic when it was administered rapidly than when it was administered slowly (Crismon *et al.*, 1943).

Toxicity. The changes in the electrocardiogram at definite concentrations of potassium in the serum both in normal and in adrenalectomized dogs indicated that the apparent toxicity of salts of potassium varied with the concentration of potassium in the serum rather than with that in the tissues. From a study of the tolerance of intact animals with a normal concentration of potassium in the muscle compared with another group in which there was a low concentration of potassium in the muscle Miller and Darrow (1940a) concluded that the toxic effects of injected potassium are directly related to the elevation of the concentration of potassium in the plasma and only indirectly to that in the muscle. Since less potassium could be removed from the plasma if the concentration in the muscle was high, it was suggested that the decreased tolerance for potassium shown by adrenalectomized animals could be explained by the high concentration of potassium in the muscle after adrenalectomy.

Zwemer (1937) suggested that potassium may be the common denominator for noxious and catabolic processes and that the increase of potassium in the plasma could be both an effect of the original stimulus and a cause of the symptoms that follow. The effects of an injection of salts of potassium on normal and adrenalectomized animals were studied and variations in the concentration of potassium in the plasma were determined (Zwemer and Truszkowski, 1936a, 1937; Truszkowski and Zwemer, 1936).

An inverse relation between the toxicity of potassium and the activity of the adrenal cortex was indicated. In the absence of the adrenal cortex tolerance to potassium was lowest and the injection of an extract of the adrenal cortex not only lowered the potassium in the blood but also protected normal animals (mice and guinea pigs) to some extent from otherwise fatal amounts of potassium administered intraperitoneally.

Schamp (1941) made strong objections to the conclusion that potassium is an important factor in adrenal insufficiency. As evidence for his contention a continuous intravenous injection of potassium chloride was made into normal conscious dogs for periods of from 2 to 12 days. No anorexia, asthenia, or convulsions were observed in any of the animals and from these data it seemed doubtful that poisoning from potassium is the primary cause of the symptoms of adrenal insufficiency in the dog.

Keith *et al.* (1942) pointed out the importance of the rate at which potassium is given. Earlier work carried out more than 100 years ago had demonstrated the toxic effect of potassium given intravenously and the absence of toxicity when a similar dose was given slowly. Large amounts of potassium salts can be ingested for the time required to reach the blood stream, the rate of excretion and the capacity of the tissues to

absorb potassium are factors which retard the accumulation of this cation in a concentration which is toxic.

Investigations on the toxicity of potassium may be divided into two groups. In the first group are studies in which a certain concentration of potassium in the serum can be related to the behavior of the heart or to survival of the animal. These concentrations of potassium in the serum are high and they obtain in adrenalectomized animals only in the terminal stages. In the second group are studies of the minimal amounts of potassium which can produce asthenia, weakness, prostration, and other symptoms of adrenal insufficiency. It is in connection with this group that controversies have arisen.

The investigations mentioned in the preceding paragraphs which come within group 2, and which were carried out on intact animals are based on an assumption the importance of which is difficult to measure. The assumption is that in the determination of the tolerance to potassium the adrenal gland of the normal experimental animal did not modify the course of the experiment. It is impossible to evaluate the degree of protection afforded by the adrenal gland of the intact animal. Valid conclusions concerning the toxicity of potassium can be made only with adrenalectomized animals that are maintained without the use of an extract of the adrenal cortex. When this was done the results indicated that the toxicity of potassium cannot be expressed in terms of its concentration in the plasma. Keith and Binger (1935) increased the concentration of potassium in the plasma of normal persons to a value higher than that observed in animals that were in a critical condition with adrenal insufficiency, but in such individuals the potassium was tolerated without untoward effect.

When adrenalectomized dogs were given a diet with high content of sodium chloride and low potassium the tolerance to potassium was decreased. After a few weeks the addition to the diet of a small amount of a potassium salt precipitated a severe prostration which terminated in death unless vigorous treatment was instituted. The least amount of potassium which was used was 0.5 g. (Kendall *et al.*, 1936; Allers *et al.*, 1936), but it was found that in the determination of the tolerance for adrenalectomized dogs it was necessary to increase the dose on each successive day (Nilson, 1937). If a constant daily dose was given the adrenalectomized dog became adapted to the intake of potassium and toxic symptoms did not appear. Adaptation to the intake of potassium occurred in the complete absence of the adrenal cortex and appeared to depend on a general systemic response.

When the adrenalectomized dog was in a state of crisis there was an increase of the concentration of potassium in the serum but this was

not sufficient to explain the severity of the prostration. Cleghorn *et al.* (1939) have confirmed and extended these observations on the toxicity of potassium.

The toxicity of potassium and the inverse relation between potassium and sodium chloride were also shown by a sudden shift in the intake of sodium chloride of an adrenalectomized dog when the diet contained a small amount of potassium. If sodium chloride was withheld from the diet the dog was prostrated and death followed within 48 hours (Kendall and Ingle, 1937).

A detailed study to explain the failure of homeostasis awaits further investigation.

Harrison and Darrow (1938) concluded that in the rat the symptoms of adrenal insufficiency were not related to changes in the distribution of water. Their results supported and amplified previous work which indicated the importance of potassium, and they pointed out that since the concentration of potassium in the cells of the intact rat is held within narrow limits the higher concentration in cases of adrenal insufficiency may be assumed to indicate important and profound changes in the muscle cell. A high intake of potassium by the rat shortened survival after adrenalectomy, and the administration of sodium chloride was followed by elimination of potassium from the blood and tissue cells and relief of the symptoms of adrenalectomy, which was just as striking as that encountered in the adrenalectomized dog with similar treatment.

The toxic effect of potassium on adrenalectomized rats was clearly shown by the use of a diet in which the content of sodium chloride was not increased but the content of potassium was greatly decreased. Wistar strain rats after adrenalectomy survived an average of 16 days on the potassium-deficient diet and an average of 11.1 days when this diet was supplemented with potassium to the normal concentration. Wisconsin strain rats after adrenalectomy survived 10.8 days on the diet low in potassium and 6.1 days when this cation was present in usual amounts (Alcott and McEwen, 1940).

Shortly after Loeb had shown the beneficial effect of sodium chloride during adrenal insufficiency Rubin and Krick (1934) suggested the use of a solution to replace tap water for treatment of adrenalectomized rats. They found from balance studies for sodium, potassium, calcium, magnesium, chlorine, phosphorus, and nitrogen that adrenal insufficiency was associated with a loss from the body of all the elements investigated. Since treatment should attempt replacement of these losses a solution of 0.7% sodium chloride, 0.035% potassium chloride, 0.0329% calcium chloride, and 0.015% magnesium chloride was substituted for the drinking water. The results were satisfactory. Adrenalectomized rats were

maintained in apparent health for 4 months with this mixture, and since that time the Rubin-Krick solution has been widely used by investigators in this field.

More recent work suggests a higher intake of sodium chloride (Anderson, 1943) and a word of caution may be in order concerning the intake of potassium. If potassium is provided in the drinking water the amount in the diet should be reduced, but since it is easier to use a diet with a normal content of potassium the need or advantage of addition of potassium to the drinking water may be questioned.

In order to separate the influence of the adrenal cortex on the tissues, quite apart from the changes induced by loss of electrolytes and water through the kidney, Ingle *et al.* (1937) determined the effect of an extract of the adrenal cortex on nephrectomized-adrenalectomized rats. The extract did not change the concentration of sodium chloride in the blood nor prevent the rise of urea, but the increase of potassium was delayed and the normal capacity of the muscle to work was maintained. The loss of muscular activity was associated with an increase of the concentration of potassium in the plasma. It was believed that the effect of the extract was concerned with its favorable effect on the distribution of potassium.

MacKay *et al.* (1937) confirmed the observations on nephrectomized-adrenalectomized rats and suggested that the most likely explanation for the result was the retention of some "toxic substance" which does not reach the serum in the presence of the adrenal glands, or is excreted in the urine when the kidneys but not the adrenals are present. They did not think that the "toxic substance" was potassium. However, it was later shown by Durlacher and Darrow (1942) that if the tissues of rats were depleted of potassium by a diet with low content of potassium, survival was lengthened after nephrectomy or ureteral ligation. There was a delay in the increase of potassium in the serum from an abnormally low value toward normal values and this delay was attributed to an uptake of potassium by the muscle. The longer survival was attributed to the delay in the rise of potassium in the plasma to toxic levels.

XI. QUALITATIVE DIFFERENCES OF STEROIDS

1. *The Sodium Factor*

Hartman and Spoor (1940) have separated a fraction from an extract of the adrenal cortex which appeared to have a specific effect on the retention of sodium, but despite continued work (Hartman and Thatcher, 1946) the chemical nature of this material has not been determined. Production of a refractory state suggests some type of sensitization to protein. Retention of sodium in normal and adrenalectomized animals

has been used as a method of standardization (Hartman *et al.*, 1941) but the use of this criterion was not recommended by Olson *et al.* (1944).

2. The Amorphous Fraction

The greatest influence on the metabolism of electrolytes is shown by the so-called amorphous fraction after separation of corticosterone and related compounds in crystalline form. Wells and Kendall (1940a) showed that when enormous doses of this fraction were given to normal rats an increase of the concentration of sodium and chloride in the serum and loss of potassium beyond the average values for intact rats did not occur. Prolonged administration of large amounts of this material did not retain sodium and chloride or produce edema.

The chemical properties of the amorphous fraction indicate the presence of an unsaturated ketone and it is probable that the active agent is a steroid. The amorphous fraction is characterized by its solubility in water, which is approximately 60 times greater than that of corticosterone. This may depend on the presence of hydroxyl groups or combination with a water-soluble moiety. Lowenstein and Zwemer (1946) suggested such a possibility.

3. Estrogens, Androgens and Related Compounds

The influence of certain steroids on the metabolism of electrolytes has been clearly shown. Talbot *et al.* (1940) found that the primary change in the uterus following an injection of estradiol was an increase of water and extracellular electrolytes. There was also an increase of the ratio of potassium to phosphate but during the following 24 hours there was rapid growth of new protoplasm and the concentration of electrolytes returned to normal values. Thorn and Engel (1938) were able to show that retention of sodium and chloride was produced by estrone, estradiol, progesterone, and testosterone propionate. Kinsell *et al.* (1942) postulated that when the cardiac glucosides are administered in small amounts their influence on the potassium in the plasma is similar to that of the hormones of the adrenal cortex.

Wood and Moe (1942) found a positive correlation between the total dose of a digitalis glucoside and the rate of increase of potassium in the serum.

Zwemer *et al.* (1940) observed similarities between the physiologic effects of the hormones of the adrenal cortex and that of some cardiac glucosides.

However, these modifications in the distribution of electrolytes are of secondary importance to the control which is exerted over the metab-

olism of sodium, potassium, and chloride by some of the steroids separated from the adrenal cortex.

4. Desoxycorticosterone

Sodium and Chloride. This 21-hydroxyl derivative of progesterone has been isolated in small amount from an extract of the adrenal cortex (Reichstein and von Euw, 1938) but the results of both chemical and physiologic investigations suggest that only traces of this compound are in the gland. The favorable effect of desoxycorticosterone on adrenalectomized dogs was first reported (Thorn *et al.* 1938) shortly after the partial synthesis of this compound had been completed (Steiger and Reichstein, 1937). Even with a low intake of sodium chloride Thorn and Eisenberg (1939) maintained an adrenalectomized dog in good condition with 1.5 mg. desoxycorticosterone acetate/day, and Cleghorn *et al.* (1941) showed that the daily dose of the compound varied inversely with the intake of sodium chloride. Desoxycorticosterone acetate given in small daily doses maintained life and growth in adrenalectomized rats when they were given purified diets virtually free of sodium chloride (Eversole, 1945).

The administration of desoxycorticosterone acetate not only stopped the loss of sodium and chloride in the adrenalectomized dog but in addition caused a marked retention of these electrolytes (Thorn *et al.* 1938).

Associated with the increase of these electrolytes in the extracellular phase there was an increase of the volume of the plasma and of the arterial pressure (reviewed by Swingle and Remington, 1944). Kuhlmann *et al.* (1939) showed that desoxycorticosterone acetate produced a marked disturbance of the metabolism of water and sodium chloride in normal dogs. Daily administration of 20–25 mg. of the steroid produced polydipsia and polyuria. Ragan *et al.* (1940) found an increased concentration of sodium and chloride in the plasma. The addition of sodium chloride to the drinking water resulted in a further increase of the intake of water and output of urine. At the same time the specific gravity of the urine was decreased. Restriction of water was followed by a sharp rise of the concentration of sodium in the serum and an increase of the specific gravity of the urine. When water was again freely allowed, the intake increased greatly, the sodium in the serum decreased and the specific gravity of the urine was less.

When the administration of desoxycorticosterone acetate was stopped, the polydipsia and polyuria disappeared even when the administration of sodium chloride was continued. Readministration of desoxycorticosterone acetate resulted in reappearance of the entire syndrome. Although the condition superficially resembled that of diabetes insipidus,

the two states were dissimilar in at least two important respects: solution of posterior pituitary was relatively ineffective and restriction of fluid did not cause dehydration. It was suggested that the most important abnormality was a thirst and secondarily a polyuria. Whether the thirst was related to the increase of sodium in the extracellular phase or to some other cause was not clear, but it was evident that the resulting polyuria enabled the normal dog to avoid excessive retention of sodium. The rapid flow of urine through the tubules would tend to counteract the increased reabsorption of sodium and chloride brought about by the desoxycorticosterone acetate.

Richter (1941) showed that in adrenalectomized rats treated with desoxycorticosterone acetate the appetite for sodium chloride decreased to the normal level. The intake of water decreased sharply during treatment and increased sharply after discontinuation of treatment. The intake of water closely followed the intake of sodium chloride.

Rice and Richter (1943) observed a different effect of desoxycorticosterone acetate in normal rats. It was found that under the influence of the steroid there was an increased appetite for salt and an associated polydipsia and polyuria. The polydipsia appeared to be a direct consequence of the high intake of sodium chloride rather than a manifestation of a primary effect on the metabolism of water.

Winter and Ingram (1943) found that in normal dogs desoxycorticosterone acetate in large doses increased glomerular filtration and reduced the reabsorption of water by the tubules. These investigators also reviewed the suggestions which have been made to explain the relation between the adrenal cortex and the pituitary body in adrenal insufficiency and in diabetes insipidus.

Potassium. Thorn *et al.* (1938) found an increase of the excretion of potassium when desoxycorticosterone acetate was given to normal dogs, and in 1939 Kuhlmann *et al.* described the striking effect of the administration of large amounts of this compound to normal dogs. There was a decrease of the concentration of potassium in the serum to values much less than normal and the dogs showed a curious periodic weakness with inability to stand or raise the head. These bouts of paralysis, except for persistence of reflexes, closely resembled familial periodic paralysis in man, which is also associated with marked decrease of potassium in the serum.

Ferrebee *et al.* (1941) extended the observations made on the loss of potassium from serum and muscle and the beneficial effect of an injection of potassium chloride on the symptoms associated with the paralysis. It was found that the ingestion of potassium chloride prevented loss of potassium from the muscle and the periodic development of weakness but the higher intake of potassium did not significantly influence either

the "diabetes insipidus" or the elevated concentration of sodium in the serum. It was therefore suggested that the "diabetes insipidus" was associated with concentration of sodium in the serum, and the muscular weakness with the concentration of sodium and potassium within the muscle cells.

Truszkowski and Duszynska (1940) reported some degree of protection afforded to mice by desoxycorticosterone acetate against the toxic action of injections of potassium chloride. For example, the L.D. 50% for normal mice weighing 9-10 g. was found to be 6.65 mg. potassium chloride/10 g. body weight. That for adrenalectomized mice was 5.75 mg./10 g. body weight, and for normal mice after injection of 1 mg. of desoxycorticosterone acetate it was 7.7 mg.

The results produced by desoxycorticosterone acetate in normal and adrenalectomized animals both on the retention of sodium and on the excretion of potassium are in strong contrast to those obtained by the administration of an extract of the adrenal cortex (Wells and Kendall, 1940a). This constitutes biologic evidence that desoxycorticosterone is not an important constituent of the secretion of the adrenal cortex.

5. Corticosterone and Related Compounds

The hormones of the adrenal cortex with a ketone or hydroxyl group at C₁₁ and with hydrogen at C₁₇ have but slight effect on the retention of sodium and chloride or on the excretion of potassium (Thorn *et al.*, 1938; Wells and Kendall, 1940a; Thorn *et al.*, 1941; Ingle *et al.*, 1946).

The two hormones with a ketone or hydroxyl group at C₁₁ and with a hydroxyl group at C₁₇ were found to increase the excretion of sodium and chloride in a normal dog (Thorn *et al.*, 1941). However, it was shown by Ingle *et al.* (1945, 1946) that the loss of sodium and chloride in the rat was but temporary and the normal balance was quickly restored.

The excretion of potassium was increased when a moderate amount of 17-hydroxycorticosterone was injected.

XII. ELECTROLYTES AND ADRENAL INSUFFICIENCY IN CLINICAL MEDICINE

1. Diagnosis

Loeb *et al.* (1937) showed that of the three important changes in a group of patients who had Addison's disease the one most frequently encountered was a decrease of the concentration of sodium in the serum. An increase of the value for potassium and an increase of nonprotein nitrogen were second and third respectively.

Harrop *et al.* (1933c) proposed a test for the diagnosis of Addison's

disease based on withholding sodium chloride from the diet of a patient for six days. However, at that time the importance of the antagonism between sodium and potassium was not realized. Wilder *et al.* (1937) showed that a patient who had adrenal insufficiency could withstand the effects of a low intake of sodium salts if the intake of potassium was also low, but if the intake of potassium was slightly increased the loss of sodium and chloride in the urine was increased and symptoms of adrenal insufficiency developed.

These observations suggested a modification of Harrop's test which was devised by Cutler *et al.* (1938) and extended by Willson *et al.* (1942). The intake of sodium chloride was limited and moderate amounts of a potassium salt were given for a period of 52 hours. Under these conditions it was found that in patients who had adrenal insufficiency the concentration of sodium and chloride in the urine during the last 4 hours of the test was so much greater than that of normal controls that the results provided a diagnostic procedure which was highly reliable.

Experience with this test disclosed the fact that the group of patients who excreted a high concentration of sodium and chloride also showed inability to excrete water in a normal manner. The volume of the urine excreted during certain intervals of the test provided information which was almost as reliable as that given by the 52-hour test (Robinson *et al.*, 1941; Levy *et al.*, 1946).

Summarizing the results of an extended study of the relation between the adrenal cortex and water diuresis and the bearing that these results have on the value of clinical diagnostic tests Gaunt (1946) expressed the following opinion: ". . . it is safe to conclude that there are no important exceptions to the rule that adrenal insufficiency, even in a relatively mild state, is associated with a decreased diuretic response to water, when water is ingested in sizable dosage."

With the results on experimental animals as a basis, Zwemer and Truszkowski (1936b) suggested a clinical test for the function of the adrenal cortex which consisted in ingestion of a salt of potassium to yield 10 mg. of potassium/pound body weight. This amount of potassium did not increase the concentration of this ion in the serum of a normal person but in cases of Addison's disease the concentration was increased. Further investigation, however, has shown that the concentration of potassium in the serum cannot be used as an index for the activity of the adrenal cortex (Greene *et al.*, 1940).

2. Treatment with Control of Electrolytes

Wilder *et al.* (1937) extended the experimental work that had established conditions satisfactory for the maintenance of adrenalectomized

dogs and showed that the same regimen was equally suitable for patients who had Addison's disease. A high intake of sodium chloride and sodium citrate was essential and it was also found that the condition of the patient was in large part dependent on the intake of potassium. As in the adrenalectomized dog the inverse relation between sodium and potassium was clearly evident. When the intake of potassium was low the intake of sodium salts could be reduced but if the intake of potassium was increased even to the amount contained in an ordinary diet sodium and chloride were rapidly lost and symptoms of adrenal insufficiency developed. With adequate control of the electrolytes the patients enjoyed a sense of well-being and carried out their usual activities indefinitely without the need of substitution therapy with an extract of the adrenal cortex.

However, the lives of these patients were in a precarious condition. Overindulgence in food with a high intake of potassium, a gastrointestinal upset, infections of the respiratory tract, a psychic strain or physical trauma, in short, anything that interfered with ingestion of sodium chloride and sodium citrate at regular intervals was a potential threat. As long as the intake of water and electrolytes remained in balance the patients were well, but in the event of a crisis the only recourse was administration of an extract of the adrenal cortex. When desoxycorticosterone became available this situation was relieved.

3. *Treatment with Desoxycorticosterone Acetate*

Thorn *et al* (1939) administered desoxycorticosterone acetate to patients who had Addison's disease and noted a positive balance of sodium and chloride, an increase of the concentration of these ions in the serum, an increased renal excretion of potassium, and an increase of the volume of the plasma, the blood pressure, and the body weight.

During the first use of desoxycorticosterone acetate it was not realized how much this compound would influence the retention of sodium and chloride. Thorn and Firor (1940) suggested that "patients with Addison's disease can be treated successfully either with desoxycorticosterone acetate alone or with this factor supplemented by sodium chloride therapy." However, they also warned that "it is evident that the quantity of supplementary sodium chloride administered to such patients must be carefully regulated during treatment with desoxycorticosterone acetate. Excessive quantities of the synthetic adrenal cortical principle and sodium chloride may result in the appearance of edema, hypertension, and in some instances signs of congestive heart failure."

It was further found that the continued administration of excessive quantities of desoxycorticosterone acetate and sodium chloride may

result in an abnormal decrease of the concentration of potassium in the serum, which in some patients was associated with muscular weakness and transient paralysis. The administration of potassium citrate was recommended to overcome the loss of potassium and restore normal muscular activity.

Ferrebee *et al.* (1939) concluded that the improvement of the condition of the patient during treatment with desoxycorticosterone acetate was qualitatively identical with that following the administration of salt and water alone, but quantitatively the improvement was far more marked.

These investigators warned against the use of salt beyond the amount in the usual diet because of the increase of the blood volume.

Tooke *et al.* (1940) also drew attention to the fact that when desoxycorticosterone acetate was used the retention of sodium and chloride and the excretion of potassium were so marked that the use of diets with the content of sodium chloride high and that of potassium low was not necessary. Von dem Borne and Lopes Cardozo (1940) warned against overdosage of desoxycorticosterone acetate, particularly with a high intake of salt.

XIII. COMMENT AND SUMMARY

The preceding sections of this review contain the evidence that the metabolism of sodium, potassium, and chloride is in large measure controlled by the hormones of the adrenal cortex. It is also true that the physiologic response to administration of the hormones of the adrenal cortex is in large measure determined by the amount of sodium and chloride present in the body. Harrison and Darrow (1938) observed that "... reduction of sodium in extracellular fluids reduces the effectiveness of the hormone. This fact was demonstrated in the experiments in which adrenalectomized rats were further depleted of sodium by intraperitoneal injection of glucose solution. It had previously been found that 5 cc. of cortical extract sufficed to produce a rapid cure of all symptoms in rats which had been allowed to progress to the terminal stages of insufficiency. However, in the adrenalectomized rats further depleted of sodium chloride, the same amount of extract merely protected the animals from prostration and immediate death since symptoms such as diarrhea and muscular weakness persisted and the blood non-protein nitrogen remained elevated. Furthermore, muscle potassium was not restored to normal as was always the case with adrenalectomized rats not further depleted of sodium and chloride."

Briefly stated, the adrenal cortex furnishes a mechanism to the body which modifies the transfer of sodium, potassium, and chloride through

cellular structures. In a normal animal deprived of salt the transfer of sodium and chloride from the glomerular filtrate to the blood against osmotic pressure until almost no sodium or chloride remains in the urine is perhaps the best example of the essential action of the adrenal cortex. This, however, is only one example. The transfer of ions through the intestinal wall, the mobilization of sodium and chloride in the extracellular compartment after depletion of these electrolytes by intraperitoneal injection of glucose, the recovery from "water intoxication" and the restoration of strength and vigor to an adrenalectomized dog that shows the typical symptoms of adrenal insufficiency solely through the administration of the hormones of the adrenal cortex without the aid of water or of a solution of sodium chloride (Swingle *et al.*, 1934c, 1936b, 1937b) are other processes which appear to depend on the same type of mechanism.

In man, the dog, or the cat in adrenal insufficiency an increase of the intake of sodium is followed by an increase of the output of potassium. The reverse is also true, but in the intact body an increase of the intake of either sodium or potassium causes but a brief increase of the output of the other ion and the normal balance is promptly restored. It would therefore seem that the hormones of the adrenal cortex modify the transfer of electrolytes in the kidney and permit this organ to perform work to overcome the direction of the ionic changes which occur in the absence of the adrenal cortex.

The remarkable effect of sodium chloride on renal function depends on something more than restoration of blood volume and of the normal concentration of electrolytes. Maintenance of renal function with sodium chloride seems of itself to have an important part in the quality of the response. However, in the absence of the adrenal cortex it is necessary to provide a high intake of sodium and chloride and thus insure a continuity of the supply of these electrolytes for the kidney. It is evident that both the conservation and continuity of supply of these essential ions are afforded by the mechanism which is furnished by the adrenal cortex.

At the present time the experimental work is still incomplete and the details of the mechanism by which electrolytes pass through cellular structures is not known. The next step appears to be progress in the field of organic chemistry, for it is only through the use of new tools that progress can come.

The availability of desoxycorticosterone has simplified the problem in several respects. In the first place it substitutes a single pure compound for the complex extract of the adrenal cortex. As a result the physiologic response is confined almost wholly to the changes in the

metabolism of sodium, potassium, and chloride and is not complicated by the influence of the C₁₁-oxygenated hormones which strongly modify the metabolism of fat, carbohydrate, and protein. Secondly, the effect of desoxycorticosterone acetate on the distribution of electrolytes and of water is of much greater magnitude than is that of any other known steroid or extract of the adrenal cortex. Finally, desoxycorticosterone has the smallest number of functional groups and therefore has the simplest possible chemical structure.

The problem can be defined at least in part in terms of organic chemistry, and although at the present stage of the investigation any suggestion must be clearly labeled as speculation, a suggestion may help to direct attention to the apparent heart of the problem. The ketol group attached to C₁₇ has been shown to be essential for the physiologic action which is inherent in desoxycorticosterone. It is possible that the ketol group, either directly or indirectly, may affect the transport of ions through cellular membranes and in this way control the distribution of electrolytes and therefore of water.

Stewart (1924) pointed out the ghost of the detoxication theory that walked through laboratories and spread confusion. This review closes with reference to a mechanism for the transport of ions by living cells. The nature of this mechanism is as elusive as a gremlin, but when it is understood we shall know how a simple group on the steroid nucleus confers physiologic freedom from domination by the electrolytes.

REFERENCES

- Alcott, D. L., and McEwen, H. D. 1940. *Proc. S. Dakota Acad. Sci.* **20**, 70-74.
 Allers, W. D. 1935. *Proc. Staff Meetings Mayo Clinic* **10**, 406-408.
 Allers, W. D., and Kendall, E. C. 1937. *Am. J. Physiol.* **118**, 87-94.
 Allers, W. D., Nilson, H. W., and Kendall, E. C. 1936. *Proc. Staff Meetings Mayo Clinic* **11**, 283-288.
 Amberg, S., and Helmholtz, H. F. 1919. *J. Pharmacol. Exptl. Therap.* **12**, 19-35.
 Anderson, Evelyn. 1943. *Essays in Biology in Honor of Herbert M. Evans*. Written by His Friends, University of California Press, Berkeley, pp. 35-49.
 Angerer, C. A., and Angerer, H. 1941. *Am. J. Physiol.* **133**, P197-198.
 Ashworth, C. T., and Gill, A. J. 1944. *Am. J. Physiol.* **142**, 435-442.
 Baetjer, A. M. 1935. *Am. J. Physiol.* **112**, 139-146.
 Banting, F. G., and Gairns, S. 1926. *Am. J. Physiol.* **77**, 100-113.
 Barnes, R. H., Miller, E. S., and Burr, G. O. 1941a. *J. Biol. Chem.* **140**, 241-246.
 Barnes, R. H., Miller, E. S., and Burr, G. O. 1941b. *J. Biol. Chem.* **140**, 247-253.
 Baumann, E. J., and Kurland, S. 1927. *J. Biol. Chem.* **71**, 281-302.
 Bavetta, L. A. 1943. *Am. J. Physiol.* **140**, 44-46.
 Bavetta, L. A., and Deuel, H. J., Jr. 1942. *Am. J. Physiol.* **136**, 712-715.
 Bavetta, L., Hallman, L. F., Deuel, H. J., Jr., and Greeley, P. O. 1941. *Am. J. Physiol.* **134**, 619-622.
 von dem Borne, G. A. K., and Lopes Cardozo, E. 1940. *Nederland. Tijdschr. Geneesk.* **84**, 231-243.

- Brewer, G., Larson, P. S., and Schroeder, A. R. 1939. *Am. J. Physiol.* **126**, 708-712.
- Britton, S. W. 1932. *Endocrinology* **16**, 633-634.
- Britton, S. W. 1939. *Am. J. Physiol.* **126**, P443-444.
- Britton, S. W., and Silvette, H. 1931. *Am. J. Physiol.* **99**, 15-32.
- Britton, S. W., and Silvette, H. 1932a. *Am. J. Physiol.* **100**, 693-700.
- Britton, S. W., and Silvette, H. 1932b. *Am. J. Physiol.* **100**, 701-713.
- Britton, S. W., and Silvette, H. 1934. *Am. J. Physiol.* **107**, 190-206.
- Britton, S. W., and Silvette, H. 1937a. *Am. J. Physiol.* **118**, 21-25.
- Britton, S. W., and Silvette, H. 1937b. *Cold Spring Harbor Symposia Quant. Biol.* **5**, 357-361.
- Britton, S. W., and Silvette, H. 1937c. *Am. J. Physiol.* **118**, 594-599.
- Britton, S. W., Silvette, H., and Kline, R. 1938. *Am. J. Physiol.* **122**, 446-454.
- Brooke, R. O., and Smith, A. H. 1933. *J. Biol. Chem.* **100**, 105-124.
- Brownell, K. A., and Hartman, F. A. 1941. *Endocrinology* **29**, 430-442.
- Buell, M. V., and Turner, E. 1941. *Am. J. Physiol.* **134**, 225-239.
- Burns, H. S., and Visscher, M. B. 1935. *Am. J. Physiol.* **110**, 490-498.
- Castleden, L. I. M. 1938. *Clin. Sci.* **3**, 241-245.
- Cattell, McK., and Civin, H. 1938. *J. Biol. Chem.* **126**, 633-644.
- Chen, G., and Geiling, E. M. K. 1943. *Proc. Soc. Exptl. Biol. Med.* **52**, 152-153.
- Cicardo, V. H., and Moglia, J. L. 1940a. *Rev. soc. argent. biol.* **16**, 54-63.
- Cicardo, V. H., and Moglia, J. L. 1940b. *Rev. soc. argent. biol.* **16**, 149-154.
- Clark, W. G. 1939. *Proc. Soc. Exptl. Biol. Med.* **40**, 468-470.
- Clark, W. G., and Barnes, R. H. 1940. *Proc. Soc. Exptl. Biol. Med.* **44**, 340-344.
- Clark, W. G., and Clausen, D. F. 1943. *Am. J. Physiol.* **139**, 70-79.
- Clark, W. G., and MacKay, E. M. 1942. *Am. J. Physiol.* **137**, 104-108.
- Clark, W. G., and Wick, A. N. 1939. *Proc. Soc. Exptl. Biol. Med.* **42**, 336-338.
- Cleghorn, R. A., Armstrong, C. W. J., and Austen, D. C. 1939. *Endocrinology* **25**, 888-898.
- Cleghorn, R. A., Fowler, J. L. A., Wenzel, J. W., and Clarke, A. P. W. 1941. *Endocrinology* **29**, 535-544.
- Cohn, C., Levine, R., and Soskin, S. 1943. *Am. J. Physiol.* **139**, 84-88.
- Cohn, C., and Soskin, S. 1943. *Am. J. Physiol.* **139**, 80-83.
- Corney, E. L. 1927. *Am. J. Physiol.* **79**, 633-640.
- Crafts, R. C. 1941. *Endocrinology* **29**, 596-605.
- Crismon, J. M., Crismon, C. S., Calabresi, M., and Darrow, D. C. 1943. *Am. J. Physiol.* **139**, 667-674.
- Cuthbertson, E. M., and Greenberg, D. M. 1945. *J. Biol. Chem.* **160**, 83-94.
- Cutler, H. H., Power, M. H., and Wilder, R. M. 1938. *J. Am. Med. Assoc.* **111**, 117-122.
- Danowski, T. S. 1941. *J. Biol. Chem.* **139**, 693-705.
- Darrow, D. C. 1944. *Ann. Rev. Physiol.* **6**, 95-122.
- Darrow, D. C., Harrison, H. E., and Taffel, M. 1939. *J. Biol. Chem.* **130**, 487-502.
- Darrow, D. C., and Yannet, H. 1935. *J. Clin. Invest.* **14**, 266-275.
- Dennis, C., and Wood, E. H. 1940. *Am. J. Physiol.* **129**, 182-190.
- Deuel, H. J., Jr., Hallman, L. F., Murray, S., and Samuels, L. T. 1937. *J. Biol. Chem.* **119**, 607-615.
- Driver, R. L. 1942. *Am. J. Physiol.* **135**, 330-337.
- D'Silva, J. L. 1934. *J. Physiol.* **82**, 393-398.
- D'Silva, J. L. 1936. *J. Physiol.* **87**, 181-188.
- Durlacher, S. H., and Darrow, D. C. 1942. *Am. J. Physiol.* **136**, 577-583.
- Evans, G. 1936. *Am. J. Physiol.* **114**, 297-308.

- Eversole, W. J. 1945. *Endocrinology* **36**, 27-31.
- Eversole, W. J., Gaunt, R., and Kendall, E. C. 1942. *Am. J. Physiol.* **135**, 378-382.
- Fenn, W. O. 1937. *Am. J. Physiol.* **120**, 675-680.
- Fenn, W. O. 1939. *Am. J. Physiol.* **127**, 356-373.
- Fenn, W. O. 1940. *Physiol. Revs.* **20**, 377-415.
- Fenn, W. O., and Cobb, D. M. 1935. *Am. J. Physiol.* **112**, 41-55.
- Fenn, W. O., and Cobb, D. M. 1936. *Am. J. Physiol.* **115**, 345-356.
- Fenn, W. O., Cobb, D. M., Manery, J. F., and Bloor, W. R. 1938. *Am. J. Physiol.* **121**, 595-608.
- Fenn, W. O., Noonan, T. R., Mullins, L. J., and Haeger, L. 1941. *Am. J. Physiol.* **135**, 149-163.
- Fenn, W. O., Wilde, W. S., Boak, R. A., and Koenemann, R. H. 1939. *Am. J. Physiol.* **128**, 139-146.
- Ferrebee, J. W., Parker, D., Carnes, W. H., Gerity, M. K., Atchley, D. W., and Loeb, R. F. 1941. *Am. J. Physiol.* **135**, 230-237.
- Ferrebee, J. W., Ragan, C., Atchley, D. W., and Loeb, R. F. 1939. *J. Am. Med. Assoc.* **113**, 1725-1731.
- Flock, E. V., Bollman, J. L., Mann, F. C., and Kendall, E. C. 1938. *J. Biol. Chem.* **125**, 57-64.
- Fowler, J. L. A., and Cleghorn, R. A. 1942. *Am. J. Physiol.* **137**, 371-379.
- Gaunt, R. 1944a. *Endocrinology* **34**, 400-415.
- Gaunt, R. 1944b. *Trans. N. Y. Acad. Sci.* **6**, 179-187.
- Gaunt, R. 1946. *J. Clin. Endocrinol.* **6**, 595-606.
- Gaunt, R., Cordsen, M., and Liling, M. 1944. *Endocrinology* **35**, 105-111.
- Gaunt, R., Remington, J. W., and Schweizer, M. 1937. *Am. J. Physiol.* **120**, 532-543.
- Gersh, I., and Grollman, A. 1939. *Am. J. Physiol.* **125**, 66-74.
- Gilman, A. 1934. *Am. J. Physiol.* **108**, 662-669.
- Greenberg, D. M., Campbell, W. W., and Murayama, M. 1940. *J. Biol. Chem.* **136**, 35-46.
- Greenberg, D. M., and Cuthbertson, E. M. 1942. *J. Biol. Chem.* **145**, 179-187.
- Greene, J. A., Levine, H., and Johnston, G. W. 1940. *Endocrinology* **27**, 375-377.
- Groat, R. A. 1941. *Am. J. Physiol.* **135**, 58-68.
- Harris, J. E. 1941. *J. Biol. Chem.* **141**, 579-595.
- Harrison, H. E., and Darrow, D. C. 1938. *J. Clin. Invest.* **17**, 77-86.
- Harrison, H. E., and Darrow, D. C. 1939. *Am. J. Physiol.* **125**, 631-643.
- Harrop, G. A. 1936a. *Bull. Johns Hopkins Hosp.* **59**, 25-34.
- Harrop, G. A. 1936b. *Bull. Johns Hopkins Hosp.* **59**, 11-24.
- Harrop, G. A., Nicholson, W. M., and Strauss, M. B. 1936. *J. Exptl. Med.* **64**, 233-251.
- Harrop, G. A., Soffer, L. J., Ellsworth, R., and Trescher, J. H. 1933a. *J. Exptl. Med.* **58**, 17-38.
- Harrop, G. A., Soffer, L. J., Nicholson, W. M., and Strauss, M. B. 1935. *J. Exptl. Med.* **61**, 839-860.
- Harrop, G. A., Jr., and Weinstein, A. 1933. *J. Exptl. Med.* **57**, 305-333.
- Harrop, G. A., Weinstein, A., Soffer, L. J., and Trescher, J. H. 1933b. *J. Exptl. Med.* **58**, 1-16.
- Harrop, G. A., Weinstein, A., Soffer, L. J., and Trescher, J. H. 1933c. *J. Am. Med. Assoc.* **100**, 1850-1855.
- Hartman, F. A., Lewis, L. A., and Thatcher, J. S. 1941. *Proc. Soc. Exptl. Biol. Med.* **48**, 60-64.

- Hartman, F. A., Smith, D. E., and Lewis, L. A. 1943. *Endocrinology* **32**, 340-344.
- Hartman, F. A., and Spoor, H. J. 1940. *Endocrinology* **28**, 871-878.
- Hartman, F. A., and Thatcher, J. S. 1946. *Federation Proc.* **5** (pt. 2), 42.
- Hastings, A. B., and Compere, E. L. 1930. *Proc. Soc. Exptl. Biol. Med.* **28**, 376-378.
- Hastings, A. B., and Eichelberger, L. 1937. *J. Biol. Chem.* **117**, 73-93.
- Hays, H. W., and Mathieson, D. R. 1945. *Endocrinology* **37**, 147-156.
- Hegnauer, A. H., and Robinson, E. J. 1936. *J. Biol. Chem.* **116**, 769-778.
- Heller, V. G., and Paul, H. 1934. *J. Biol. Chem.* **105**, 655-661.
- Helve, O. E. 1940. *Biochem. Z.* **306**, 343-398.
- Heppel, L. A. 1939. *Am. J. Physiol.* **127**, 385-392.
- Heppel, L. A. 1940. *Am. J. Physiol.* **128**, 449-454.
- Holten, C., and Rehberg, P. B. 1931. *Acta Med. Scand.* **74**, 479-518.
- Houssay, B. A., and Marenzi, A. D. 1937. *Rev. soc. argent. biol.* **13**, 139-151.
- Ingle, D. J. 1940. *Am. J. Physiol.* **129**, 278-282.
- Ingle, D. J., Nilson, H. W., and Kendall, E. C. 1937. *Am. J. Physiol.* **118**, 302-308.
- Ingle, D. J., and Oberle, E. A. 1946. *Am. J. Physiol.* **147**, 222-227.
- Ingle, D. J., Sheppard, R., Evans, J. S., and Kuizenga, M. H. 1945. *Endocrinology* **37**, 341-356.
- Ingle, D. J., Sheppard, R., Oberle, E. A., and Kuizenga, M. H. 1946. *Endocrinology* **39**, 52-57.
- Ingraham, R. C., and Visscher, M. B. 1936a. *Am. J. Physiol.* **114**, 676-680.
- Ingraham, R. C., and Visscher, M. B. 1936b. *Am. J. Physiol.* **114**, 681-687.
- Joseph, M., Cohn, W. E., and Greenberg, D. M. 1939. *J. Biol. Chem.* **128**, 673-683.
- Joseph, S., Schweizer, M., Ulmer, N. Z., and Gaunt, R. 1944. *Endocrinology* **35**, 338-346.
- Keith, N. M., and Binger, M. H. 1935. *J. Am. Med. Assoc.* **105**, 1584-1590.
- Keith, N. M., Osterberg, A. E., and Burchell, H. B. 1942. *Ann. Internal Med.* **16**, 879-892.
- Kendall, E. C. Unpublished data
- Kendall, E. C., Flock, E. V., Bollman, J. L., and Mann, F. C. 1938. *J. Biol. Chem.* **126**, 697-708.
- Kendall, E. C., and Ingle, D. J. 1937. *Science* **86**, 18-19.
- Kendall, E. C., Mason, H. L., Myers, C. S., and Allers, W. D. 1936. *J. Biol. Chem.* **114**, lvii-lviii.
- Keys, A. 1938. *Am. J. Physiol.* **121**, 325-330.
- Kinsell, L. W., Zilleson, F. O., Smith, A. M., and Palmer, J. 1942. *Endocrinology* **30**, 221-228.
- Koneff, A. A., Holmes, R. O., and Reese, J. D. 1941. *Anat. Record* **79**, 275-289.
- Kottke, F. J., Code, C. F., and Wood, E. H. 1942. *Am. J. Physiol.* **136**, 229-243.
- Kuhlmann, D., Ragan, C., Ferrebee, J. W., Atchley, D. W., and Loeb, R. F. 1939. *Science* **90**, 496-497.
- Kutz, R. L., McKeown, R., and Selye, H. 1934. *Proc. Soc. Exptl. Biol. Med.* **32**, 331-332.
- Larson, P. S. 1940. *Am. J. Physiol.* **130**, 562-567.
- Laszt, L. 1939. *Nature* **144**, 244.
- Laszt, L., and Verzá, F. 1936. *Biochem. Z.* **288**, 351-355.
- Levy, M. S., Power, M. H., and Kepler, E. J. 1946. *J. Clin. Endocrinol.* **6**, 607-632.
- Light, A. E., Smith, P. K., Smith, A. H., and Anderson, W. E. 1934. *J. Biol. Chem.* **107**, 689-695.
- Loeb, R. F. 1932. *Science* **76**, 420-421.
- Loeb, R. F. 1933. *Proc. Soc. Exptl. Biol. Med.* **30**, 808-812.

- Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J. 1933. *J. Exptl. Med.* **57**, 775-792.
- Loeb, R. F., Atchley, D. W., and Parson, W. 1937. *Trans. Assoc. Am. Physicians* **52**, 228-235.
- Loeb, R. F., Atchley, D. W., and Stahl, J. 1935. *J. Am. Med. Assoc.* **104**, 2149-2154.
- Long, C. N. H., Katzin, B., and Fry, E. G. 1940. *Endocrinology* **26**, 339-344.
- Lowenstein, B. E., and Zwemer, R. L. 1946. *Endocrinology* **39**, 63.
- Lucas, G. H. W. 1926. *Am. J. Physiol.* **77**, 114-125.
- MacKay, E. M. 1937. *Am. J. Physiol.* **120**, 361-364.
- MacKay, E. M., and Barnes, R. H. 1937. *Am. J. Physiol.* **118**, 184-189.
- MacKay, E. M., Bergman, H. C., and MacKay, L. L. 1937. *Am. J. Physiol.* **120**, 83-86.
- Manery, J. F., and Bale, W. F. 1941. *Am. J. Physiol.* **132**, 215-231.
- Manery, J. F., and Haeger, L. F. 1941. *Am. J. Physiol.* **134**, 83-93.
- Manery, J. F., and Hastings, A. B. 1939. *J. Biol. Chem.* **127**, 657-676.
- Marine, D., and Baumann, E. J. 1927. *Am. J. Physiol.* **81**, 86-100.
- Marshall, E. K., Jr., and Davis, D. M. 1916. *J. Pharmacol. Exptl. Therap.* **8**, 525-550.
- Martin, S. J., Herrlich, H. C., and Fazekas, J. F. 1939. *Am. J. Physiol.* **127**, 51-57.
- Mellors, R. C., Muntwyler, E., and Mautz, F. R. 1942a. *J. Biol. Chem.* **144**, 773-784.
- Mellors, R. C., Muntwyler, E., Mautz, F. R., and Abbott, W. E. 1942b. *J. Biol. Chem.* **144**, 785-793.
- Miller, H. C., and Darrow, D. C. 1940a. *Am. J. Physiol.* **130**, 747-758.
- Miller, H. C., and Darrow, D. C. 1940b. *Am. J. Physiol.* **129**, 264-270.
- Miller, H. C., and Darrow, D. C. 1941. *Am. J. Physiol.* **132**, 801-809.
- Mullin, F. J., Dennis, J., and Calvin, D. B. 1938. *Am. J. Physiol.* **124**, 192-201.
- Muntwyler, E., Mellors, R. C., and Mautz, F. R. 1940a. *J. Biol. Chem.* **134**, 345-365.
- Muntwyler, E., Mellors, R. C., Mautz, F. R., and Mangun, G. H. 1940b. *J. Biol. Chem.* **134**, 367-387.
- Nilson, H. W. 1937. *Am. J. Physiol.* **118**, 620-631.
- Noonan, T. R., Fenn, W. O., and Haeger, L. 1941. *Am. J. Physiol.* **132**, 474-488.
- Olson, R. E., Jacobs, F. A., Richert, D., Thayer, S. A., Kopp, L. J., and Wade, N. J. 1944. *Endocrinology* **35**, 430-455.
- Orent-Keiles, E., and McCollum, E. V. 1940. *J. Biol. Chem.* **133**, 75-81.
- Orent-Keiles, E., and McCollum, E. V. 1941. *J. Biol. Chem.* **140**, 337-352.
- Orent-Keiles, E., Robinson, A., and McCollum, E. V. 1937. *Am. J. Physiol.* **119**, 651-661.
- Parkins, W. M., Hays, H. W., and Swingle, W. W. 1936. *Am. J. Physiol.* **117**, 13-23.
- Parkins, W. M., Taylor, A. R., and Swingle, W. W. 1935. *Am. J. Physiol.* **112**, 581-590.
- Peters, H. C. 1941. *Am. J. Physiol.* **134**, 37-39.
- Peters, J. P. 1935. *Body Water; the Exchange of Fluids in Man*, Charles C Thomas, Springfield, Illinois.
- Ponder, E., and Gaunt, R. 1934. *Proc. Soc. Exptl. Biol. Med.* **32**, 202-204.
- Pulver, R., and Verzár, F. 1940. *Helv. Chim. Acta* **23**, 1087-1100.
- Ragan, C., Ferrebee, J. W., Phyfe, P., Atchley, D. W., and Loeb, R. F. 1940. *Am. J. Physiol.* **131**, 73-78.
- Rapoport, S., and Guest, G. M. 1942. *Proc. Soc. Exptl. Biol. Med.* **49**, 147-149.

- Reichstein, T., and von Euw, J. 1938. *Helv. Chim. Acta* **21**, 1197-1210.
- Remington, J. W. 1940. *Endocrinology* **26**, 631-640.
- Remington, J. W. 1943. *Endocrinology* **32**, 129-134.
- Rice, K. K., and Richter, C. P. 1943. *Endocrinology* **33**, 106-115.
- Richter, C. P. 1936a. *Endocrinology* **20**, 657-666.
- Richter, C. P. 1936b. *Am. J. Physiol.* **115**, 155-161.
- Richter, C. P. 1939. *Endocrinology* **24**, 367-371.
- Richter, C. P. 1941. *Endocrinology* **29**, 115-125.
- Richter, C. P., and Eckert, J. F. 1938. *Endocrinology* **22**, 214-224.
- Robinson, E. J., and Hegnauer, A. H. 1936. *J. Biol. Chem.* **116**, 779-786.
- Robinson, F. J., Power, M. H., and Kepler, E. J. 1941. *Proc. Staff Meetings, Mayo Clinic* **16**, 577-583.
- Rogoff, J. M., and Stewart, G. N. 1928. *Am. J. Physiol.* **84**, 649-659.
- Rowntree, L. G. 1926. *J. Pharmacol. Exptl. Therap.* **29**, 135-159.
- Rubin, M. I., and Krick, E. T. 1934. *Proc. Soc. Exptl. Biol. Med.* **31**, 228-229.
- Sandberg, M., Perla, D., and Holly, O. M. 1937. *Endocrinology* **21**, 352-356.
- Schamp, H. M. 1941. *Endocrinology* **29**, 459-466.
- Schwartz, B. M., Smith, P. K., and Winkler, A. W. 1942. *Am. J. Physiol.* **137**, 658-670.
- Selkurt, E. E., and Houck, C. R. 1944. *Am. J. Physiol.* **141**, 423-430.
- Shannon, J. A. 1936. *Am. J. Physiol.* **117**, 206-225.
- Shipley, R. A. 1945. *Endocrinology* **36**, 118-123.
- Silvette, H. 1934. *Am. J. Physiol.* **108**, 535-544.
- Silvette, H., and Britton, S. W. 1932. *Am. J. Physiol.* **100**, 685-692.
- Silvette, H., and Britton, S. W. 1935. *Am. J. Physiol.* **111**, 305-311.
- Silvette, H., and Britton, S. W. 1936. *Am. J. Physiol.* **115**, 618-626.
- Silvette, H., and Britton, S. W. 1938. *Am. J. Physiol.* **121**, 528-533.
- Smith, A. H., and Smith, P. K. 1934. *J. Biol. Chem.* **107**, 681-688.
- Smith, D. E., Lewis, L. A., and Hartman, F. A. 1943. *Endocrinology* **32**, 437-442.
- Smith, H. W. 1937. *The Physiology of the Kidney*, Oxford University Press, New York.
- Smith, H. W. 1943. *Lectures on the Kidney*, University Extension Division, University of Kansas, Lawrence, Kansas.
- Somogyi, J. C., and Verzá, F. 1941. *Arch. intern. pharmacodynamie* **65**, 221-248.
- Spoor, H. J., and Ralli, E. P. 1944. *Endocrinology* **35**, 325-337.
- Steiger, M., and Reichstein, T. 1937. *Helv. Chim. Acta* **20**, 1164-1179.
- Stein, L., and Wertheimer, E. 1941. *Proc. Soc. Exptl. Biol. Med.* **46**, 172-174.
- Steinbach, H. B. 1940. *J. Biol. Chem.* **133**, 695-701.
- Stewart, G. N. 1924. *Physiol. Revs.* **4**, 163-190.
- Stewart, G. N., and Rogoff, J. M. 1924-25. *Proc. Soc. Exptl. Biol. Med.* **22**, 394-397.
- Stickney, J. C. 1941. *Am. J. Physiol.* **132**, 9-17.
- Swingle, W. W., Parkins, W. M., and Taylor, A. R. 1936a. *Am. J. Physiol.* **116**, 430-437.
- Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W. 1936b. *Am. J. Physiol.* **116**, 438-445.
- Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W. 1937a. *Am. J. Physiol.* **119**, 557-566.
- Swingle, W. W., Parkins, W. M., Taylor, A. R., and Hays, H. W. 1937b. *Am. J. Physiol.* **119**, 684-691.
- Swingle, W. W., Pfüfner, J. J., Vars, H. M., and Parkins, W. M. 1934a. *Am. J. Physiol.* **108**, 159-167.

- Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M. 1934b. *Am. J. Physiol.* **108**, 428-437.
- Swingle, W. W., Pfiffner, J. J., Vars, H. M., and Parkins, W. M. 1934c. *Am. J. Physiol.* **108**, 144-150.
- Swingle, W. W., and Remington, J. W. 1944. *Physiol. Revs.* **24**, 89-127.
- Swingle, W. W., Remington, J. W., Hays, H. W., and Collings, W. D. 1941. *Endocrinology* **28**, 531-534.
- Talbot, N. B., Lowry, O. H., and Astwood, E. B. 1940. *J. Biol. Chem.* **132**, 1-9.
- Thaddea, S. 1935. *Z. ges. expil. Med.* **95**, 600-626.
- Thorn, G. W., and Eisenberg, H. 1939. *Endocrinology* **25**, 39-46.
- Thorn, G. W., and Engel, L. L. 1938. *J. Expil. Med.* **68**, 299-312.
- Thorn, G. W., Engel, L. L., and Lewis, R. A. 1941. *Science* **94**, 348-349.
- Thorn, G. W., and Firor, W. M. 1940. *J. Am. Med. Assoc.* **114**, 2517-2525.
- Thorn, G. W., Howard, R. P., Emerson, K., Jr., and Firor, W. M. 1939. *Bull. Johns Hopkins Hosp.* **64**, 339-365.
- Thorn, G. W., Lewis, R. A., and Eisenberg, H. 1938. *J. Expil. Med.* **68**, 161-171.
- Tooke, T. B., Jr., Power, M. H., and Kepler, E. J. 1940. *Proc. Staff Meetings, Mayo Clinic* **15**, 365-368.
- Truszkowski, R., and Duszynska, J. 1940. *Endocrinology* **27**, 117-124.
- Truszkowski, R., and Zwemer, R. L. 1936. *Biochem. J.* **30**, 1345-1353.
- Verzár, F. 1939. Die Funktion der Nebennierenrinde, Benno Schwabe and Co., Basel.
- Verzár, F., and Laszt, L. 1936. *Biochem. Z.* **288**, 356-358.
- Visscher, M. B., Fetcher, E. S., Jr., Carr, C. W., Gregor, H. P., Bushey, M. S., and Barker, D. E. 1944b. *Am. J. Physiol.* **142**, 550-575.
- Visscher, M. B., Varco, R. H., Carr, C. W., Dean, R. B., and Erickson, D. 1944a. *Am. J. Physiol.* **141**, 488-505.
- Wells, B. B., and Kendall, E. C. 1940a. *Proc. Staff Meetings, Mayo Clinic* **15**, 133-139.
- Wells, B. B., and Kendall, E. C. 1940b. *Proc. Staff Meetings, Mayo Clinic* **15**, 565-573.
- Wilder, R. M., Kendall, E. C., Snell, A. M., Kepler, E. J., Rynearson, E. H., and Adams, M. 1937. *Arch. Internal Med.* **59**, 367-393.
- Wiley, F. H., and Wiley, L. L. 1933. *J. Biol. Chem.* **101**, 83-92.
- Willson, D. M., Robinson, F. J., Power, M. H., and Wilder, R. M. 1942. *Arch. Internal Med.* **69**, 460-469.
- Winkler, A. W., Hoff, H. E., and Smith, P. K. 1938. *Am. J. Physiol.* **124**, 478-483.
- Winkler, A. W., Hoff, H. E., and Smith, P. K. 1941. *Am. J. Physiol.* **133**, P494-495.
- Winkler, A. W., and Smith, P. K. 1938. *J. Biol. Chem.* **124**, 589-598.
- Winter, C. A., and Ingram, W. R. 1943. *Am. J. Physiol.* **139**, 710-718.
- Wood, E. H., and Moe, G. K. 1942. *Am. J. Physiol.* **137**, 6-21.
- Wood, E. H., Collins, D. A., and Moe, G. K. 1940. *Am. J. Physiol.* **128**, 635-652.
- Zwemer, R. L. 1934. *Endocrinology* **18**, 161-169.
- Zwemer, R. L. 1937. *Cold Spring Harbor Symposia Quant. Biol.* **5**, 323-325.
- Zwemer, R. L., Lowenstein, B. E., and Pines, K. L. 1940. *Endocrinology* **27**, 945-955.
- Zwemer, R. L., and Sullivan, R. C. 1934. *Endocrinology* **18**, 97-106.
- Zwemer, R. L., and Truszkowski, R. 1936a. *Science* **83**, 558-560.
- Zwemer, R. L., and Truszkowski, R. 1936b. *Proc. Soc. Expil. Biol. Med.* **35**, 424-426.
- Zwemer, R. L., and Truszkowski, R. 1937. *Endocrinology* **21**, 40-49.

Cumulative Index of Vols. I-V

A

- Abortion,
 - habitual, II, 108
 - maternal diet and, III, 80, 95
 - phosphorus deficiency and, III, 77
 - vitamin deficiency and, III, 81, 88, 91
 - vitamin E and, II, 129
- Absorption,
 - gastrointestinal, impaired, in sprue, V, 124
- Absorption spectra,
 - of *Avena*, I, 209
 - enzymes, I, 109, 115
 - homovitamin A ethyl ether, V, 9
 - hormones, I, 109, 115
 - Phycomyces*, I, 209
 - single nerve fibers, V, 99
 - Spirillum rubrum*, I, 202
 - vitamin A derivatives, V, 9
 - vitamin A homologues, V, 9
 - vitamins, I, 109, 115, 127, 132
- Acetobacter suboxydans*,
 - p-aminobenzoic acid and II, 239; III, 173, 174, 177, 180
 - oxidation of inositol by, III, 209
 - pantoic lactone requirement of, III, 155
 - purines and, III, 201
- 2-Acetylaminofluorene,
 - tumors arising from, V, 279
- Acetylcholine, I, 1, 5, 11, 13, 16; III, 337 ff.
 - amounts released during action potential of electric organ, III, 363
 - "cycle," III, 360
 - effect of eserine on liberation of, III, 371, 372
 - on ganglia, III, 369
 - formation of, I, 41, 42
 - intracellular action, I, 368
 - nerve excitation and liberation of, V, 94-96
 - in perfusion fluid, I, 369-371
 - synthesis of, I, 360, 365-368
 - thiamine and, III, 367; V, 96, 109, 113
- Acetylcholinobufagin,
 - crystal structure of, II, 442
- 3-Acetylpyridine, effect on mice, V, 192
- Achlorhydria, I, 283, 286
 - in Addisonian anemia, I, 97
 - in pernicious anemia I, 270
 - in nutritive failure, I, 80
- Achromia,
 - pernicious anemia and, I, 270
- Achromotrichia, III, 55; V, 184
 - p-aminobenzoic acid and, II, 220
 - hormones and, II, 219
 - L. casei* factor and, III, 67
 - melanin pigment in, II, 217
 - metal deficiency and, II, 219
 - sulfonamides and, III, 54, 57
 - vitamins and, II, 35, 38, 219, 220, 223; III, 30, 57, 58
- Acids, dental caries and, II, 255, 270, 271, 276, 290, 299
- Acne,
 - effect of androgens on, V, 334
- Acrodynia,
 - pyridoxine and, I, 92
- Acromegaly,
 - effect of androgens on, V, 376, 377
 - estrogens on, V, 376, 377
- Actiniasterol,
 - crystal structure of, II, 450, 451, 454, 455
- Action potential,
 - acetylcholine released during, of electric organ, III, 363
 - and diffusion potentials, III, 359
 - of nerve, III, 353, 359, 360
 - parallelism between choline esterase concentration and voltage of, in electric organ, III, 356, 358, 359
- Addison's disease,
 - electrolyte balance in, IV, 290, 291
 - treatment with steroids, IV, 266, 271, 272, 274, 276, 285; V, 377, 378
- Adenine,
 - absorption spectrum, I, 132, 137, 138
 - antagonism to benzimidazole, III, 203, 204
 - bacterial growth and, III, 199-201
- Adenylic acid, I, 166, 264
 - effect on hemolytic streptococci, III, 141, 200

- Adipic acid,
as degradation product of biotin, II,
49, 51, 68
- Adrenal cortex,
carcinoma of, I, 311
dysfunction of,
effect of androgens on, IV, 265, 271,
274, 275
estrogens on, IV, 276, 277
extracts,
 $\Delta^4,5$ -pregnadiene-17, 21-diol-3, 11, 20
trione in, I, 337
hormones, see under Adrenocortical
hormones and under name of
individual hormones
isolation of steroids from, I, 337, 348
pseudohypophysectomy and, IV, 136
role of, in the conversion of progesterone to pregnanediol, I, 304, 305
as source of androgens, I, 314; V, 222
stimulation by pituitary extracts, I,
329
vitamins in, I, 177, 242
- Adrenal glands,
aerobic respiration rate in, I, 239
anaerobic glycolysis rate in, I, 239
excretion of androgens in women and,
I, 308
effect of androgens on, V, 267
corticotrophic hormone on, V, 226 ff.
estrogens on V, 262, 263
on glucose formation, IV, 202
goitrogenic agents on, V, 295
hyperplasia,
in mice following ovariectomy, I, 329
urinary excretion of sodium pregnanediol glucuronide in
women with, I, 304
of steroids in persons with, I, 309,
311
necrosis of, III, 55
sulfonamides and, III, 57
removal, see Adrenalectomy
as source of steroids, I, 297, 299, 302,
304, 326, 327
vitamins and, I, 177, 236-238, 240, 241,
242, 245; IV, 148, 151
- Adrenalectomy,
corticosterone administration after, I,
362
growth and survival after, I, 346, 347;
IV, 348-352; V, 208
- Adrenaline,
effect on tyrosinase activity, II, 232
hyperglycemic effect of, IV, 192
thyroid and sensibility to, IV, 192
vitamins and, I, 157, 176-178
- Adrenocortical hormones (see also under
name of individual hormones), I, 295,
345-413
assay of, IV, 335-358
unit of, IV, 343
Adrenocorticotrophic hormone,
see Corticotrophic hormone
Adrenosterone (Δ^4 -androstene-3,11,17-
trione), I, 365, 368; II, 362
formation in vivo, I, 314
isolation of, I, 308
structure of, I, 313, 373; II, 363
- Adsorbents,
effect on vitamin B absorption, V, 65
- Aerobacter aerogenes*,
vitamin synthesis by, III, 28, 30
- Agar, effect on resorption of gonadotropin, III, 323
- Agglutinin, IV, 56, 63
protein deficiency and formation of,
IV, 63
vitamin deficiency and production of,
IV, 63
- Agranulocytosis, sulfonamides and, IV,
22
- β -Alanine, III, 151 ff.
requirement of *Corynebacterium diphtheriae*, III, 152
of yeast, II, 30; III, 152
- β -Alanine betaine,
lipotropic activity and, I, 29
- Albumin (see also Egg white, Serum),
growth factors in egg, I, 263, 264
nutrition and serum, I, 98, 99
- Alcaligenes faecalis*,
vitamin synthesis by, III, 28-30
- Algae, I, 196, 201-204
carotenoids in, I, 196
photoactive systems of, I, 211
photosynthesis in, I, 201
vitamins in, I, 195, 232
- Allergy,
effect of vitamins on, I, 173, 174
food, I, 80, 81
liver extract injections and, 282
- Allocholesterol,
as growth factor for *Trichomonas columbae*, I, 261
crystal structure of, II, 447
- Allopregnane, I, 235, 296, 351-354, 358-
360, 364, 365, 367, 373, 377
- 3(α),20(α)-Allopregnanediol,
isolation of, I, 298
as metabolite of progesterone, I, 317
urinary excretion of, I, 301

- 3(β),20(α)-Allopregnenediol,
isolation of, I, 298
as metabolite of progesterone, I, 317
urinary excretion of, I, 301
- Allopregnenedione,
isolation of, I, 299
as metabolite of progesterone, I, 317
- 3,17,20,21-Allopregnanetetrol, I, 380
- 3(α),16,20-Allopregnanetriol, see Preg-
nanetriol B
- Allopregnanolone,
isolation, I, 297
- Allopregnanol-3(α)-one-20,
isolation, I, 299
as metabolite of progesterone, I, 317,
319
- Allopregnanol-3(β)-one-20,
isolation, I, 299
as metabolite of progesterone, I, 317,
319
- Δ^1 -Allopregnene-3,17-dione,
yeast and, I, 322
- Alloxan,
effect on islet cells, IV, 192
thyroid diabetes, IV, 196
thyroidectomy and, IV, 202, 203
- Alloxazine-adenine-dinucleotide, I, 171
- Allylthiourea,
effect on cytochrome oxidase, V, 291
muscle, 299
goitrogenic activity of, V, 275
as inhibitor of thyroxine formation,
V, 289
tumors arising from, V, 279
- Alopecia, III, 55
inositol and, III, 30, 52, 63, 67; V, 189
in mice, III, 207
sulfonamides and, III, 54, 57
vitamins and, I, 93; III, 57, 67; V,
186, 189
- Aluminum salts, effect on resorption of
gonadotropin, III, 323
- Amboceptor,
dietary deficiency and production of,
IV, 63
- d*-Amino acid oxidase,
effect of vitamins on, II, 337
inhibition of, II, 339
in liver, II, 326
- Amino acids, I, 182
antagonism to sulfonamides, III, 178
aromatic,
effect on cytochrome oxidase, V, 291
derivatives of, I, 158, 186, 187
fetus and, IV, 76
functions of, I, 158
metabolism, I, 158, 161
proteins and, IV, 75
vitamins and, I, 157-159, 161, 162,
164, 166, 180, 183, 185, 187; III,
7; IV, 77, 101
- Aminoazobenzene,
riboflavin in hepatomas produced by,
II, 316
- Aminoazotoluene,
depigmentation caused by, II, 220
liver tumors induced by, II, 310, 322,
323, 331, 334
- Aminobenzene compounds,
effect on iodine, V, 287, 300
goitrogenic activity of, V, 277, 289
relative activities of, V, 303
- p*-Aminobenzoic acid, II, 215-246; III,
63, 174, 176; IV, 5, 6
as amphicarcinogen, II, 344
in animal tissue, I, 229
antagonism to sulfonamides, III, 34,
53, 54, 56, 66, 68, 69, 172, 175,
178-180, IV, 22, 23
antibacterial activity of, II, 235
antithyroid activity of, V, 315
antivitamins of, II, 342
assay of, III, 174, 177, 180
as bacterial growth factor, III, 173,
174, 177
bile acids and absorption of, I, 162
bile salts and, IV, 75
biological synthesis of, III, 34, 77; V,
72
as chromotrichia factor, I, 93, 94
determination of, II, 240-242
detoxifying action of, II, 227, 228
effect on achromotrichia, II, 223-225
basal metabolic rate, V, 284
lactation, II, 223, IV, 140, 141
melanin formation, V, 292
microorganisms, II, 233-236
oxidation of *p*-cresol, I, 171
of tyrosine, I, 171
- growth and, II, 221-223, 238, 239; III,
14; IV, 139
inositol and, III, 33; V, 78
phagocytosis and deficiency of, IV, 64
physicochemical properties of, II, 236-
239
physiological role of, II, 245, 246
pigmentation and, II, 216-221
purines and, III, 201, 204-206
relation to enzymes, II, 230-232
to hormones, II, 228-230
reproduction and, IV, 139
requirement of chick, V, 168

- sources of, II, 242, 243
- specificity of, III, 174
- toxicity of, II, 226, 227
- vitamin M deficiency and, II, 91
- Aminohexose, in antipernicious anemia substance, III, 253
- p-Aminomethylbenzenesulfonamide (marfanil), III, 174
- p-Aminophenols,
 - p-aminobenzoic acid and, II, 228
 - in urine, II, 338
- p-Aminophenylacetic acid, III, 174
 - thyroxine formation and, V, 288
- Aminopyrimidines,
 - absorption spectrum, I, 128
- Amphibians,
 - effect of goitrogenic agents on metamorphosis of, V, 315
- Amphicarcinogens, II, 344
- Anahaemin, III, 252, 290
- Anasarca,
 - vitamin deficiency and, IV, 88, 91
- "Androgen-estrogen ratio," V, 420, 421
- Androgens,
 - activity of, II, 376, 380, 382
 - adrenal cortex as source of, V, 222
 - bioassay of, V, 319-330
 - chemistry of, II, 362 ff.
 - clinical use of, V, 339-382
 - contraindications to, V, 417
 - effect on birds, II, 361-408
 - blood acid phosphatase, II, 355
 - of vitamin E on metabolism of, II, 327
 - on women, V, 325-339, 380
 - effects of, V, 334, 338, 339, 359
 - estrogens and, IV, 151, 161, 166, V, 235, 263-267
 - excretion of, V, 235, 395
 - homosexuality and, V, 418-422
 - inactivation of, IV, 149, 151, 154, 166
 - international standard, II, 379
 - lactation and, V, 369
 - modes of administration, V, 319-325, 411-416
 - structure of, II, 363, 364
 - therapeutic use of, IV, 161, 166; V, 411-416, 422-425
 - urinary, IV, 154
- Δ^1 - Δ^2 -Androstadien-17-one,
 - isolation of, I, 311, 312
- Androstane,
 - structure of, II, 363
- 3(α),17(α)-Androstanediol,
 - effect on electrolyte balance, IV, 292
 - metabolic effects of, IV, 275
 - renotropic effect of, IV, 300
 - structure of, IV, 258
- 3-cis-17-trans-Androstanediol, II, 362
 - effect on birds, II, 366, 376, 380, 381, 382, 384, 385
 - structure, II, 363
- Androstanediol-3-benzoate,
 - activity of, II, 380
 - effect on birds, II, 365, 369
 - relation between configuration and activity of, II, 381
- Androstane-3(β),11-diol-17-one,
 - isolation of, I, 308
- 3,17-Androstanedione, II, 362
 - activity of, I, 380, 382
 - mode of administration and activity of, II, 381
 - structure of, II, 363
- Androstan-3(β)-ol-7-one,
 - isolation of, I, 309
- Androstan-17-ol-3-one (dihydrotestosterone),
 - structure of, II, 362
- Androstan-3-ol-17-ones,
 - see Androsterone and Isoandrosterone
- Δ^5 -Androstene-3,17-diol,
 - conversion of dehydroisoandrosterone to, I, 325
 - oxidation of, I, 326
- Δ^5 -Androstene-3(β),17(α)-diol,
 - effect on nitrogen excretion, V, 262, 274
 - structure of, V, 258
- Androstenediols, II, 362
 - activity of, II, 380
 - and configuration, II, 381
 - effect on birds, II, 364, 385
 - structure, II, 363; IV, 258
- Androstenedione,
 - absorption spectrum, I, 137
 - estrogenic activity of, I, 337
- Δ^1 -Androstene dione-3,17, pr. crs.
 - reduction by yeast, I, 319, 325
- Δ^4 -Androstene-3,17-dione, I, 369; II, 259-261
 - in adrenal cortex, I, 308
 - from Δ^5 -androstene-3,17-diol, I, 326
 - conversion to androsterone, I, 316, 318
 - to testosterone, I, 325
 - effect on blood urea, II, 278
 - nitrogen excretion, II, 261
 - synthesis of, II, 259
- Δ^5 -Androstene-3,17-dione,
 - as metabolite of testosterone, I, 319
- Androstenediones, II, 362
 - activity of, II, 380
 - configuration and, II, 381

- assay of, II, 376
 effect on birds, II, 364
 structure of, II, 363
 3,16,17-Androstenetriol,
 isolation, I, 311
 Androsten-3(α)-ol-17-one,
 isolation, I, 310, 311
 Δ^1 -Androsten-17-ol-3-one,
 from Δ^1 -androstenedione-3,17, I, 325
 Δ^4 -Androsten-3(β)-ol-17-one,
 see Dehydroisoandrosterone
 Androstenolones,
 structure of, II, 363, 364
 Androstenone,
 isolation, I, 310, 312
 Androsterone, II, 362
 absorption spectrum, I, 119
 activity of, II, 379, 380
 configuration and, II, 381
 from Δ^4 -androstenedione, I, 316
 assay of, I, 138, 151
 colorimetric determination, I, 144
 conversion to testosterone, I, 315
 crystal structure of, II, 436, 438, 440
 dose response curves, II, 377, 383
 effect on birds, II, 365 ff., 374, 376, 379
 estrogens and, V, 264, 267
 isolation of, I, 308-310
 metabolic effects of, V, 273
 as metabolite of progesterone, I, 316-318
 structure of, II, 363
 in urine, I, 311
 iso-Androsterone, II, 362
 activity of, II, 380
 configuration and, II, 381
 structure of, II, 363
 Androsterone acetate,
 mode of administration and activity
 of, II, 381
 Androsterone benzoate,
 effect on birds, II, 365
 mode of administration and activity
 of, II, 381
 Anemia, III, 55
 Addisonian, I, 97, 283, 284
 in children fed goat's milk, V, 154
 congenital, III, 80
 effect of liver and liver extracts on, I, 246; II, 93
 factor R and, IV, 17, 18
 factor S and, IV, 19
 hemoglobin in, II, 95
 hemorrhagic,
 L. casei factor and, IV, 24
 hypochromic,
 ascorbic acid and, I, 174
 effect of, in mothers on fetus, III, 80
 vitamin B₆ and, IV, 24
 infantile, I, 95
 iron deficiency and, I, 62-64
 L. casei factor and, III, 61, 62, 67
 macrocytic, I, 97, 269-288; II, 92, 94
 liver extract and, IV, 2, 13
 response to pteroylglutamic acid,
 IV, 141
 vitamin B₆ and, IV, 8, 12, 13
 macrocytic, hyperchromic,
 vitamin M and, IV, 19
 in malnutrition, I, 98
 maternal diet and, III, 80, 92, 98
 microcytic, I, 98, 174
 nutritional,
 in infants, IV, 119-121
 nutritional, macrocytic,
 effect of pteroylglutamates on, V, 122
 of vitamin M on, V, 139 ff.
 similarity to vitamin M deficiency,
 V, 140
 sprue and, V, 139 ff.
 symptoms of, V, 13
 pernicious, I, 269-288; III, 238 ff.
 effect of thiamine on, V, 145, 154
 pathogenesis of, V, 150
 of pregnancy, V, 140
 sprue and, V, 141
 therapeutic value of liver extract in,
 V, 142, 153
 of pteroylglutamates in, V, 122-124, 142-150, 153
 pteroylglutamic acid deficiency in, V, 152
 pyracin and, IV, 18, 19
 refractory,
 pteroylglutamic acid and, V, 121
 in scorbutic guinea pigs, I, 174
 in sprue, I, 95
 sulfonamides and, III, 54, 62
 temporary pernicious, of infancy,
 criteria of, V, 153
 nutritional origin of, V, 153
 therapeutic value of vitamin "B₆" in,
 III, 62
 tropical, I, 282, 287
 types of, I, 286
 vitamin deficiency and, IV, 106, 142
 Anestrus,
 avitaminosis B and, IV, 148, 151
 gonadotropic hormone and, IV, 144
 underfeeding and, IV, 144
 Aneurine, see Thiamine

- Angina pectoris**,
 androgen therapy of, V, 422-425
- Anol**,
 crystalline, III, 233
 estrogenic activity of, III, 232
 polymers of, III, 232
- Anorexia**,
 in nutritive failure, I, 73, 80, 81, 84,
 91, 94, 177
- Anthraquinones**, III, 215
- Antianemia factor**, nutritional, IV, 1-19
- Antianemia liver fraction**, IV, 178
- Antibiotin**,
 avidin and, IV, 140
 egg white and, IV, 139
- Antibiotin factor**, III, 169, 170
 antagonism to biotin, III, 18, 99, 170
 antibacterial action of, III, 169, 170
- Antibodies**,
 nutrition and formation of, IV, 63
 vitamin deficiency and formation of,
 IV, 63
- Antibody titers**,
 corticotropic hormone and, V, 226
- Anticarcinogens**, II, 344
- Antidermatitis factor**, identity with
 pantothenic acid, III, 29
- Antigonadotropin (antiprolan)**, III, 298,
 313, 314, 317, 320
- Anti-insulin effect**, I, 357
- Antimony trichloride**,
 determination of vitamin A with, V,
 43
 as reagent for retinenes, I, 216, 219
 for vitamin A, I, 144, 219
 spectra of reaction products of, with
 pigments, I, 215, 217, 218
 test, I, 134
- Antioxidant**, tocopherol as, III, 3, 14
- Antipellagra vitamin**, see Niacin
- Antipernicious anemia factor**, I, 97, 269-
 291
- Antipernicious anemia substance**, III,
 237 ff.
 accessory factors to, III, 265-268,
 274-278, 281-283, 291
 aminohexose in, III, 253
 analyses of, III, 244, 246, 247, 249,
 253, 254, 259, 262, 266, 267, 269,
 285, 286
 glutathione as, III, 284
 hydrolysis of, III, 251, 253, 254, 255,
 269
 liver, as source of, III, 237 ff.
 multiple factor hypothesis, III, 265
 peptide nature of, III, 254-256, 258
 primary factor of, III, 265 ff.
 pterine nature of, III, 279
 quinine salt of, III, 251
- Antiprolan**, see Antigonadotropin
- Antiprotease**, see Heparin
- Antiserum for gonadotropin**, see Anti-
 gonadotropin
- Antithiamine factor**, III, 129-132, 170
- Antithyroid agents** (see also Goitrogenic
 agents),
 biology of, V, 274-307
- Antunit**, definition of, III, 300
- Antivitamins**, II, 342
- Antivitamins**, V, 82
 in natural foods, V, 71
 and vitamin deficiencies, V, 71
- Apocarotenals**,
 physical properties, II, 185
- Appetite**,
 vitamins and, I, 161, 172
- Arachidonic acid**, II, 8, 19-22
 effect on fat deficiency syndrome, III,
 76
- Arachin**,
 in diets, I, 33, 41, 50, 182
 non-lipotropic action of, I, 20
- Arginase**,
 ascorbic acid and, I, 171, 183
 effect of corticotropic hormone on, V,
 222
 of steroids on, IV, 301
- Arginine**,
 testis degeneration and, II, 146
- Ariboflavinosis**,
 manifestations of, I, 88, 101, IV, 93-95
 in mice, V, 180, 181
 tryptophan and, IV, 94
- Arsenicals**,
 detoxification by p-aminobenzoic acid,
 II, 218, 228
- Arsenocholine**,
 lipotropic properties, I, 48
- Arteries**,
 effect of ovarian hormones on, V, 339
- Arthropods**,
 appearance of image-forming eyes in,
 I, 196, 197, 215, 224
 retinenes and vision in, I, 224
 vitamin A and vision in, I, 214, 219,
 221
- Ascorbic acid (vitamin C)**, I, 34, 60, 75,
 94, 116, 131, 138; II, 71, 79-86, 90,
 322-324; IV, 100-106
 absorption of, IV, 100
 absorption from placenta, III, 89
 absorption spectrum of, I, 130

- amino acids, and, IV, 77, 101
 as antioxidant for carotene, III, 3
 assay of, II, 322
 biological synthesis of, III, 23
 bone formation and, III, 9
 capillary resistance and, III, 4
 caries and, II, 80
 choline and, III, 8
 colorimetric determination of, I, 177
 correlation with respiratory activity
 of tissues, I, 238, 239, 245
 daily allowances of, I, 76, 82
 dark adaptation and, III, 5, 6, 17
 deficiency, I, 77, 94, 162, 185; III, 88,
 89
 abortion and, III, 88
 manifestations of, II, 82 ff., IV, 103,
 104
 phagocytosis and, IV, 64, 65
 relation to other diseases, IV, 106, 107
 dentition and, II, 83, 93
 determination of, IV, 103, V, 43
 effect of corticotropic hormone on,
 in adrenals, V, 226 ff.
 on cytochrome oxidase, V, 291
 on reproduction, III, 88, 89, 92
 on teeth, III, 9
 on tumors, II, 316, 322-324, 328
 excretion of, V, 52, 63
 fetus and, III, 88, 89, 94
 function of, IV, 100, 101
 hyperkeratoses and, III, 11
 interrelation with citrin, III, 4
 with other vitamins, II, 9; III, 2-3,
 8, 14, 16, 17
 metabolism of, II, 328; IV, 100
 relief of toxic effects by, III, 16
 requirements, III, 89; IV, 102; V, 189,
 190
 hesperidin and, III, 4
 orange peel extract and, III, 4
 resistance to infection and, IV, 50, 57
 sources of, II, 82, 323; IV, 101-103
 tyrosine and deficiency of, V, 81
 utilization of, II, 328
 visual purple synthesis and, III, 4
 Ascorbic acid oxidase, I, 171, 178, 179;
 V, 62
 Asparagine,
 requirements of microorganisms for,
 I, 253, 255
 Aspartic acid,
 lipotropic activity and, I, 19
 Astacene,
 absorption spectrum, I, 212
 Astaxanthine,
 absorption spectrum, I, 212, 213
 distribution of, I, 212, 213, 219, 223,
 224
 Asthma,
 effect of p-aminobenzoic acid on, II,
 225, 229
 Atmospheric pressure,
 corticotropic hormone and resistance
 to low, V, 225
 Atrophy,
 muscular,
 pyridoxine deficiency and, I, 93
 spinal, progressive,
 effect of vitamin E on, I, 97
 Atropine,
 effect on "vagus-stoff," III, 338
 Auximer, II, 342
 Auxin(s),
 biotin as, II, 33
 as growth stimulants, I, 265
 Avidin, see also Antibiotin factor
 in animal nutrition, II, 34
 antibiotin and, IV, 139
 anticarcinogenic activity of, II, 344
 biotin and, I, 157, 167, 235; II, 36, 313,
 342
 in egg white, I, 167; II, 34
 formation of, II, 33
 growth inhibitory effect of, I, 168
 progesterone and, IV, 140
 stilbestrol and, IV, 140
 Avidin-biotin complex,
 reproduction and, IV, 140
 Avitaminoses,
 effect on nerve tissue, V, 114
 manifestations of, I, 65, 176; II, 202,
 203; V, 111
 nitrogen metabolism and, I, 162, 163,
 187
 Axon,
 peripheral, excitable properties of, III,
 340
 of squid, choline esterase in giant, III,
 350
 thiamine in, III, 367
 Axoplasm,
 choline esterase in, III, 350
 respiratory enzymes in, III, 351, 358
Azotobacter chroococcum,
 effect on growth of wheat seedlings,
 III, 25
Azobacter, coenzyme-R synthesis by, III,
 165
 Azonaphthalene,
 depigmentation due to, II, 220
 Azoöspemia, V, 410

p-Azoxyanisole, II, 428
p-Azoxyphenetole, II, 428

B

- Bacillus acidophilus odontolyticus*,
dental caries and, II, 274, 275
Bacillus adhaerens,
thiamine synthesis by, III, 28
Bacillus anthracis,
glutamine requirement, III, 146
Bacillus diphtheriae,
pimelic acid as nutrient for, II, 37
Bacillus dysenteriae,
uracil requirement of, IV, 199
vitamin A deficiency and resistance to,
IV, 199
vitamins B and, IV, 28, 181, 185, 191,
193, 196, 199
Bacillus mesentericus,
vitamin synthesis by, III, 28, 29
Bacillus proteus, see *Proteus vulgaris*
Bacillus subtilis,
thiamine synthesis by, III, 28
Bacillus vulgaris, see *Proteus vulgaris*
Bacillus vulgatus,
vitamin synthesis by, III, 28-30
Bacteria,
ascorbic acid in, I, 232, 233
avidin-biotin complex and, I, 168
classification of, III, 186, 187
dental caries and, II, 265, 274, 279, 281
drug resistance of, III, 179
effect on metabolism of steroid hor-
mones, I, 295, 317, 320-323, 325
growth factors for, I, 263; III, 141, 200,
212
effect on metabolism of steroid hor-
mones, I, 295, 317, 320-323, 325
of vitamins on, II, 98, 199; III, 139
growth factors for, I, 263, III, 139, 141,
200, 212
growth of,
inhibitory action of iodinin on, III,
215
initiation of, III, 147
nicotinamide and, III, 181-183,
186 ff.
uracil and, III, 198-201
growth-stimulating substances pro-
duced by, III, 24, 25
intestinal,
death due to lack of, III, 24
effect of sulfonamides on, III, 66;
IV, 22
growth and, III, 24
vitamin B₆ and, IV, 10
lipides in, II, 2
metabolism,
thiamine and, III, 119, 120
nutritional deficiency and resistance
to, IV, 46
pigments of, I, 223
synthesis of vitamins by, I, 176; III,
23-48, 50, 52, 54, 60, 66, 68, 96,
120, 135-139, 143, 164, 179 ff.,
190-192, 214
of inositol, III, 23, 30, 35, 52, 69
of tetrahydroquinone, III, 209
of uracil, III, 198
vitamin content of, I, 232, 233, 244;
II, 33, 324
Bacteriochlorophyll,
absorption spectrum of, I, 202, 204, 205
Bacteriostasis, III, 195
produced by iodinin, III, 215
by sulfonamides, III, 24, 43, 44, 53
Bacterium coli, see *Escherichia coli*
Batyl alcohol,
vitamin A activity of, II, 167
Benzimidazole,
action of yeasts on, III, 203
antagonism to adenine, III, 203, 204
to guanine, III, 203
to purines, III, 204
and biotin, III, 204
as growth inhibitor, III, 203
Benzopyrene,
carcinogenetic activity of, II, 354
oxidation by ascorbic acid, II, 324
structural similarity with estrogenic
substances, II, 358
tumors and, II, 311, 318, 319, 326
Beriberi,
cardiac, I, 100, 101
congenital, III, 84, 98
deaths from, I, 61
incidence among rice eaters, III, 39
manifestations of, I, 72, 73; III, 10;
V, 88-91
in monkeys, II, 86-88, 102
nutrition and, II, 87
pre-anatomic stage of, I, 62
prevalence of, I, 60
pyruvic acid and, IV, 88
riboflavin and, III, 134
thiamine and, I, 72; II, 88; III, 84, 118;
IV, 88, 89
Betaine,
antihemorrhagic action of, I, 34, 50,
181
determination of, I, 11, 12, 14, 15

- growth-promoting activity of, I, 25, 45
 lipotropic activity of, I, 18, 29, 42, 46, 47
 utilization of homocysteine and, I, 180
- Bile,**
 choline in, I, 2-4, 38
 effect on vitamin absorption, I, 162, 163; IV, 79, 80
- Bile acids,**
 effect on digestion of fats, I, 158
 formation from cholesterol, I, 335, 336
 oxidation of, I, 322
- Bile pigments,**
 from hemoglobin, I, 175, 186
 increased excretion of, I, 279
- Bile salts,**
 effect on availability of vitamin A, V, 58
 on resorption of gonadotropin, III, 323
 on vitamin absorption, I, 162, 163
 vitamin K and, IV, 115
- Bios, II, 30**
- Biotin, see also Coenzyme R, II, 26-69;**
 III, 63 ff., 158 ff.
 absorption spectrum of, I, 116, 132
 achromotrichia and, II, 35, 218
 alopecia and, III, 57
 p-aminobenzoic acid and, II, 239, 243
 as anticarcinogen, II, 313
 assay of, III, 121, 164, 165
 avidin and, I, 167, 247; II, 34, 37, 313, 314, 342, 344; III, 99
 benzimidazole and, III, 204
 biological synthesis, V, 72
 carcinogenetic activity of, I, 40
 chemistry of, II, 40-55
 cholesterol and, I, 44
 chromotrichia and, III, 62
 correlation coefficient between aerobic respiration and, I, 238, 239
 anaerobic glycolysis and, I, 238, 239
 α -Biotin,
 between niacin content of tissues, I, 235
 deficiency, I, 94
 fetal resorption and, IV, 139
 resistance to bacterial infection and, IV, 55, 56
 degradation of, III, 167, 170
 determination of, II, 31, 32
 distribution of, I, 234; II, 32, 33, 313, 335-337
 effect on dermatitis, III, 54, 57
 on "egg-white injury," III, 57
 on embryonic tissues, II, 36, 314
 on fungi, III, 166
 on porphyrin caking, III, 54, 57
 on prothrombin, III, 56
 estrogens and, IV, 138
 excretion of, III, 30
 fat metabolism and, I, 32; II, 3, 4, 6, 9-13, 36
 growth activity of, I, 258; IV, 2
 identity with vitamin H, III, 160, 169
 inactivation of, II, 37
 insect nutrition and, III, 166
 interrelation with other vitamins, III, 3, 7, 32, 58, 63
 intestinal synthesis of, II, 345; III, 30, 33-34
 isolation of, II, 37-40
 lactation and, IV, 141
L. casei factor and, III, 63
 methyl ester, II, 31, 32, 37-41, 50, 61; III, 160, 168
 occurrence in animal tissues, I, 229, 233, 236, 238, 239
 in bacteria, I, 234, 244
 in human tissues, I, 237
 in lower organisms, I, 234, 244
 oxidation by rancid fats, III, 3
 requirements, affected by avidin, III, 18, 33
 of bacteria III, 163, 166, 209
 of fungi, III, 33, 169
 of mice, V, 188
 of poultry, V, 167, 169, 170, 171
 of yeasts, III, 159-162
 role in animal nutrition, II, 33-35
 human nutrition, II, 35
 specificity of, II, 36, 37
 sulfonamides and, II, 34; III, 29-30, 34, 54, 56, 57, 66, 68; IV, 23, 24; V, 74
 tumors and, II, 36, 313, 314, 332, 340, 346
 vitamers of, II, 314
- α -Biotin,
 degradation of, II, 66
 sources of, II, 66
- β -Biotin, sources of, II, 66
- Biotin-avidin complex, V, 74
- Birds,**
 effect of androgens on II, 361-408
 of estrogens on, II, 361-408
 of goitrogenic agents on, V, 280, 281, 285
- Birth,**
 premature,
 maternal diet and, III, 95
 weight,

- maternal nutrition and, III, 74-76, 92
- Bismuth salts,
effect on resorption of gonadotropin, III, 323
- Bixin, II, 162
- Bladder,
effect of androgens on, V, 337, 426
- Blindness,
maternal vitamin A deficiency and congenital, III, 81, 83
from optic nerve constriction, III, 82
- Blood,
p-aminobenzoic acid in, II, 226, 241
analysis of, I, 184, 271
cocarboxylase in, IV, 86
coenzyme I and, IV, 98
effect of steroids on phosphorus of, IV, 113
on proteins of, IV, 279
of vitamin deficiencies on, II, 17; III, 28, 67, 84
goitrogenic agents and, V, 297, 298
as growth factor for flagellates, I, 261
iron in, IV, 119
in malnutrition, I, 97, 98
non-protein nitrogen of, I, 164
oxygen content of, I, 271
in pernicious anemia, I, 269, 270
urea content, I, 347
vitamin K and coagulation of, IV, 58
vitamins in, I, 98; IV, 80, 81, 87, 95, 98, 101, 142
- Blood plasma,
ascorbic acid content of fetal, III, 89
effect of dietary calcium on calcium content of, III, 77
- Blood sugar,
thyroid and, IV, 188, 189, 198, 199
- Blood vessels,
calcifications of, III, 63, 67
- Bone ash,
effect of Ca/P ratio on, III, 78
- Bone marrow,
action of goitrogenic agents on, V, 298
cobalt and respiration of red cells in, I, 174
hypocellularity of, III, 55, 67
pernicious anemia and, I, 270, 271, 275, 284
reticulocyte production by, I, 278
sulfonamides and, III, 54
- Bones,
development in fetus, IV, 108
phosphatase and regeneration of, V, 213
- in rickets, IV, 112, 113
in scurvy, IV, 107
vitamins and, IV, 41, 100, 109
- Brain,
aerobic respiration in, I, 239
anaerobic glycolysis in, I, 239
antipernicious anemia principle in, I, 283
choline in, I, 3, 4
choline esterase in, III, 345, 346
effect of copper deficiency on, III, 79
of pantothenic acid on, III, 88
of prenatal starvation on, III, 74
of thiamine deficiency on, V, 179
succinoxidase in, I, 166
vitamin content of, I, 232, 236, 244
- Brassica* seeds,
adrenocortical hypertrophy caused by, V, 295
effect of dietary, on maturation of ovary, V, 296
goitrogenic activity of, V, 276
- Brassica* species,
goitrogenic activity of, V, 276
- Brassicasterol, II, 454, 455
- Breast, see also Mammary gland
effects of androgens on, V, 334, 372
functional and pathological disorders of, V, 364-373
pathophysiology of, V, 364
- Breeding,
effect of liver on, III, 87
of phosphorus deficiency on, III, 77
of vitamins on, III, 77, 87, 88
- Bromomethoxyestrone,
crystal structure of, II, 438
- Brucella* spp.,
effect of pantothenic acid on, III, 155
- Bufagin,
crystal structure of, II, 441, 442
empirical formula, II, 445
molecular weight, II, 445
- Butter yellow, see also Dimethylamino-azobenzene, I, 165
carcinogenetic activity of, I, 39, 165
demethylation of, I, 40
hepatic injury due to ingestion of, I, 36, 38, 165, 182
tumors arising from, IV, 164
- Butyric acid, II, 14, 275
biological formation, II, 16

C

- Calciferol (see also Vitamin D₂), II, 20
crystal structure of, II, 435, 454, 455

- structure of, II, 457
- therapeutic effect on dermatitis, II, 21
- Calciferol-pyrocalfiferol,
 - crystal structure of, II, 435, 456
- Calcium, I, 176
 - absorption from intestines, I, 95
 - in blood, II, 76
 - blood coagulation and, I, 176
 - breast milk and, IV, 107
 - deficiency,
 - effect on activity, III, 76
 - on food consumption, III, 76
 - on growth, III, 76
 - on life span, III, 76
 - in monkeys, II, 75, 79
 - on paralysis, III, 76
 - on sensitivity, III, 76
 - on spontaneous fractures, III, 77
 - deposition of, IV, 107, 110
 - dental caries and, I, 63
 - dietary, II, 72, 76, IV, 94, 95
 - and blood plasma, IV, 77
 - and growth, IV, 77
 - and vitamin D requirement, IV, 18
 - in flour, I, 79
 - metabolism of, IV, 95
 - effect of vitamin A on, II, 202
 - vitamin D on, IV, 107, 108
 - non-diffusible, in spasmophilia, IV, 110
 - reproduction and, IV, 76-78, 92, 108
 - requirements, I, 70, 75-77, 82
 - in serum, I, 98, 103
 - tetany and, IV, 113
- Calcium/phosphorus ratio, III, 89
 - effect on bone ash, 78
 - on fetal rickets, 78
 - on gestation, 78
 - on lactation, 78
 - on rickets, 89
- Cancer, see Carcinoma
- Capillaries,
 - ascorbic acid and, III, 4
 - vitamin K and, IV, 117
 - vitamin P and, III, 4
- Caprylin, II, 18
- Capsanthin,
 - reaction with antimony trichloride, II, 162
 - vitamin A activity of, II, 165
- Carbohydrates,
 - dental caries and, I, 63; II, 278-299
 - in diet, I, 69, 164
 - effect on bacterial vitamin synthesis,
 - III, 31, 38, 39, 44
 - on contracted pelvis, III, 75
 - on reproduction, III, 76
 - on vitamin requirements, III, 42
 - fat metabolism and, II, 1, 2, 8, 19
 - glycogen and, II, 19
 - intake of, and urinary thiamine output, V, 64
 - metabolism of, I, 347, 348; II, 24, 147
 - corticotrophic hormone and, V, 224, 225
 - vitamins and, I, 158; II, 17, 18; III, 6, 8; IV, 86, 91
 - pregnancy and, III, 92
 - refection and, V, 66
 - synthesis of riboflavin and, V, 68
 - vitamin B complex deficiency and appetite for, I, 161
 - vitamins and digestibility of, I, 162
 - and oxidation of, III, 17
- Carbon dioxide,
 - as growth factor for bacteria, III, 141, 200
- Carcinogenesis,
 - butter yellow and, I, 38-40, 165
 - factors influencing, I, 38-40, 165
 - isolation of hydrocarbons inducing, I, 143
 - promotion of, by estrogens, I, 295
 - protective effect of riboflavin, I, 165
- Carcinogens, II, 314
 - definition of, II, 354
 - detoxification of, II, 327
 - effect of vitamin A on, II, 311
 - estrogenic substances and, II, 358
 - sex hormones and, II, 353, 354
- Carcinoma, see also Tumors and under name of individual neoplasms
 - agents producing, I, 38
 - castration as treatment for, II, 354
 - diabetes and, I, 80
 - estrogens and, II, 354, 357, 358; IV, 167
 - food intake and, I, 80
 - isolation of steroids from patients with, I, 309-311
 - role of hormones in, II, 359
 - transmission of mammary, II, 358
 - vitamins and, I, 86, 246; II, 306, 311, 315, 316, 319, 321, 323, 327, 328, 343, 345
- Caries, dental, II, 255-298
 - carbohydrates and, I, 63
 - chemo-parasitic theory of, II, 268-274, 276
 - enamel structure and, II, 272, 281-283
 - etiology of, II, 255-298
 - inhibitory effect of fluorine on, II, 256, 282, 285-298

- of saliva on, II, 279-281
- microorganisms and, II, 268-279
- in rats, II, 256, 263-268
- recording of, II, 257-263
- role of nutrition, II, 283-285
- vitamin D and, III, 9; IV, 109
- Carnosine, I, 158
- Carotenase, II, 163
 - carotene and, IV, 79
- Carotene,
 - absorption spectrum of, I, 134; II, 166
 - activity of, II, 161 ff; 204
 - antioxidants for, III, 3
 - assay of, II, 162
 - bile and absorption of, IV, 79
 - in blood, IV, 81, 83
 - carcinoma and, II, 328
 - carotenase and, IV, 79
 - chemistry of, II, 163-197
 - conversion to vitamin A, II, 162, 198, 205
 - dietary and plasma, in pregnancy, III, 82
 - distribution of, I, 107, 196, 197, 207, 223, 230; II, 115; IV, 81, 83
 - effect on enzymes, I, 162, 171
 - on eyes, I, 88
 - on fat metabolism, II, 7
 - empirical formula, I, 199
 - excretion of, II, 204
 - interrelation with vitamin A, I, 71; IV, 79
 - with other vitamins, III, 13, 14
 - intestinal absorption of, II, 204
 - oxidation of, III, 3
 - physical properties, II, 172
 - in plant photokinetics, I, 223, 224
 - separation from vitamin A, II, 194
 - sources, II, 159, 194, 195, 197, 198; III, 14
 - therapeutic value in dermatitis, II, 21
- α -Carotene,
 - absorption spectra of, I, 118, 135
 - assay of, I, 138, 151
 - properties of, II, 163, 165
 - structure of, II, 164
 - vitamin A activity of, II, 165, 177, 194
- β -Carotene,
 - absorption spectra of, I, 118, 135, 204, 205
 - assay of, I, 138, 151
 - degradation of, II, 184-186
 - distribution of, I, 196, 207, 208, 230
 - effect on vitamins, II, 145, 194
 - properties of, II, 163-165, 173
 - vitamin A activity of, II, 164, 165, 188
- γ -Carotene,
 - absorption spectrum of, I, 118, 138
 - activity of, II, 165
 - assay of, I, 138
 - properties of, II, 163-165
- β -Carotene oxide,
 - properties of, II, 165
 - structure of, II, 164
 - vitamin A activity of, II, 165
- Carotenoids,
 - absorption spectra of, I, 203-207, 209-211, 215
 - biological activity of, I, 222; II, 160-161, 202
 - chemistry of, I, 99, 167, 198, 199, 218
 - cis-trans isomerism in, V, 9, 32
 - determination in blood, IV, 80
 - distribution of, I, 5, 198, 208, 212, 219, 223, 230
 - photoreceptor function of, I, 195, 197, 225
 - and photosynthesis, I, 200-202
 - precursors of, I, 134
 - properties of, II, 161, 165
 - retinas and, I, 186, 216, 231
 - proteins and, I, 157, 166, 206, 215, 216, 223
- Carotin, I, 212
- Cartilage,
 - effect of fasting on, III, 75
 - growth hormone on, V, 213, 214
 - vitamins on, III, 86, 88; V, 213, 214
- Cascin,
 - antagonism to p-dimethylaminobenzene, II, 332
 - as anticarcinogen, II, 344
 - B vitamins and, I, 161; II, 9
 - biological value of autoclaved, I, 164
 - dietary, and sulfonamides, III, 55, 56
 - effect of choline on, II, 6
 - on resorption of gonadotropin, III, 323
 - estrus cycle and, III, 76
 - extrinsic factor in, I, 286
 - intake of, I, 182
 - lipotropic effect of, I, 18-24, 28, 42
 - and liver diseases, I, 36, 37, 39, 45, 49, 165, 181
 - therapeutic value in dermatitis, I, 22
- Castrates,
 - gonadotropin titer of, V, 408
- Castration,
 - effect on birds, II, 371, 376, 395
 - on blood phosphatase, II, 355
 - on pituitary, V, 250, 326
 - gonadotropins and, V, 398, 408

- homosexuality and, V, 422
- as treatment for carcinoma, II, 354
- Canities,
 - melanin pigment in, II, 217
- Catecholase,
 - action of p-aminobenzoic acid on, II, 233
- Cats,
 - effect of estrogens on, V, 258
- Cecum,
 - bacterial synthesis of vitamins in, III, 26, 27, 29, 30, 35
 - effect of dextrinized starch diet on, III, 26
 - vitamin K deficiency and removal of, III, 56
- Celiac disease, V, 58
 - response to pteroylglutamic acid, V, 153
- Cell membrane,
 - esterine and permeability of, III, 372
- Cells,
 - effect of vitamins on, II, 145, 309, 322, 325
- Cereals,
 - dental caries and, II, 264, 268, 293-299
 - vitamin E in germs of, II, 115
- Cervisterol,
 - crystal structure of, II, 435, 454, 455
- Cheilosis,
 - in diabetes, IV, 173
 - riboflavin deficiency and, IV, 93
- Chemical transmitter theory, of nerve, III, 340, 360
- Chicken,
 - p-aminobenzoic acid requirement of, V, 168
 - asymmetry of gonads in, II, 365
 - choline requirement of, V, 167, 168, 170, 171
 - effect of androgens on, II, 366
 - of sterile environment on, III, 24
 - symptoms of vitamin deficiency in, II, 122
 - vitamin requirements of, II, 143; V, 169
- Child,
 - androgens and the preadolescent, V, 417
- Chimyl alcohol,
 - vitamin A activity of, II, 167
- 3-Chloroandrostanone,
 - crystal structure of, II, 438
- 3-Chloro-androsten-17-one,
 - isolation from urine, I, 312
- α -Chlorocholestane,
 - crystal structure of, II, 450
- Chlorophyll,
 - absorption spectrum of, I, 202, 205, 206
 - carotenoids and, I, 196-203, 210
 - effect on resorption of gonadotropin, III, 323
 - stability of, I, 198
- Chlorophyll A,
 - absorption spectrum of, I, 202, 204, 205
 - carotenoids as precursors of, I, 199
 - empirical formula, I, 197
 - in tomatoes, I, 200
- Chlorophyll B, I, 204, 205
 - absorption spectrum, I, 204, 205
 - carotenoids as precursors for, I, 199
 - empirical formula, I, 197
 - in tomatoes, I, 200
- Chloroplast,
 - absorption spectrum of pigments of, I, 201
 - carotenoids as constituents of, I, 196
 - orientation, I, 203, 209
- Chloroplastin, I, 198
- Chlorothiamine, III, 125
- Cholanic acid,
 - crystal structure of, II, 447
- Choleic acid,
 - crystal structure of, II, 428
- Cholestane,
 - crystal structure of, II, 435
- epi-Cholestane,
 - crystal structure of, II, 447
- α -Cholestanetriol,
 - crystal structure of, II, 435
- Cholesterol,
 - crystal structure of, II, 440, 447, 450, 452
 - as growth factor for *Trichomonas columbae*, I, 261
- epi-Cholesterol,
 - crystal structure of, II, 440, 450, 452
- Cholestan-6-ol-ethyl alcohol,
 - crystal structure of, II, 435
- Cholestanone, effect of yeast on, I, 322
- Cholestene,
 - crystal structure of, II, 435
- α -Cholestene dibromide,
 - crystal structure of, II, 447
- β -Cholestene dibromide,
 - crystal structure of, II, 435
- cis- $\Delta^{5,6}$ -Cholestene-3,4-diol,
 - crystal structure of, II, 435
- Cholestene hydrochloride,
 - crystal structure of, II, 435, 447
- Δ^4 -Cholesten-7-ol,

- crystal structure of, II, 433, 435, 436
- Cholestenone,
 conversion of cholesterol to, I, 323
 crystal structure of, II, 452, 453
 effect of bacteria on, I, 322
- Δ^1 -Cholestenone,
 effect of yeast on, I, 322
- Cholesterol,
 antilipotropic effect of, I, 47, 50
 bacteria and, I, 322
 biosynthesis of, I, 337; II, 1, 12, 13, 132
 in body fat, II, 14
 configuration, II, 450
 crystal structure of, II, 435, 447, 451, 452
 effect of choline on, I, 32, 34, 42, 44, 45; II, 12
 corticotropic hormone on, in adrenals, V, 226 ff.
 fatty livers and, II, 3
 as growth factor for protozoa, I, 251, 259, 261, 263
 hypercholesterolemia after feeding, I, 42
 metabolism of, I, 18-20; II, 132
 vitamin E and, II, 327
 steroids from, I, 322, 323, 335, 336, 358, 359; V, 227
 vitamin B complex and formation of, II, 14
- epi-Cholesterol,
 crystal structure, II, 447
- Cholesterol glycol, I, 323
- Cholesterol-gonadotropin pellets, III, 302, 303
- Cholesterol methyl alcohol,
 crystal structure of, II, 435
- Cholesteryl acetate,
 crystal structure of, II, 435
- Cholesteryl bromide, II, 448, 450
 crystal structure of, II, 428-433, 436
- Cholesteryl chloride, II, 450
 crystal structure of, II, 428, 432, 433, 448
- Cholesteryl chloride hydrochloride,
 configuration of, II, 499
 crystal structure of, II, 449
- Cholesteryl iodide, II, 458, 459
 configuration of, II, 448
 crystal structure of, II, 429, 431-433
- Cholesterylene,
 crystal structure of, II, 435, 437
- Cholic acid, I, 162
- Choline, I, 1-58; II, 3, 4, 6; III, 63; IV, 54, 57; V, 78, 79
 acetylation of, III, 363; V, 115
- as amphicarcinogen, II, 344
- antihemorrhagic action of, I, 48
- availability of, V, 78
- casein and, I, 46; II, 6
- cholesterol and, I, 42, II, 12, 340
- deficiency,
 effect of niacin on, III, 8
 on maturity, III, 88
 on reproduction, III, 88
 phagocytosis and, IV, 64
- determination of, I, 9, 10, 11
- in diet, II, 219
- distribution of, I, 2, 10, 11, 13; II, 6
- effect on dermatitis, II, 22
 on growth, I, 41
 on kidney, II, 338
 on lactation, I, 42; IV, 140, 141
 on liver, II, 3, 6, 11
 on perosis, I, 41, 182
- enzymes and, I, 13
- estrogens and, IV, 138
- factor R and, IV, 17
- fat metabolism and, I, 158, 180
- as growth factor for *Neurospora*, III, 212
 for *Pneumococcus*, III, 210, 211
- lipotropic action of, I, 41; II, 11, 12, 21
- methylation of homocysteine by, I, 180, 186
- requirement for, V, 170, 171, 189
- as toxic agent in cod liver oil, III, 16
- tumors and, II, 307, 321, 330, 333
- vitamin characteristics of, I, 51, 159, 160, 166
- Choline derivatives,
 activity of, III, 211
- Choline acetylase, III, 365 ff.
 dialysis of, III, 366
 effect of citric acid on, III, 357
 of cysteine on, III, 367
 of fluoride on, III, 366
 of glutamic acid on, III, 366, 367
 of iodoacetate on, III, 366
 of K on, III, 366
- inhibition by Cu, III, 366
- requirement of *Diphlococcus pneumoniae*, III, 210, 211
- Choline esterase, III, 341 ff.
 affinity to strychnine, III, 372
 concentration in electric organ, III, 356, 358
 at neuromuscular junction, III, 341-344
 in spinal cord, III, 345, 358
 at synapses, III, 344-350
- Chondrification,

- effect of riboflavin deficiency on, III, 88
- Chondrogenesis,
effect of growth hormone on, V, 200
- Chondromucin,
ascorbic acid and deposition of, I, 169
- Chromans, antisterility activity of, II, 120
- Chromatin,
vitamin E and synthesis of nuclear, II, 146
- Chromotrichia,
biotin and, III, 62
folic acid and, III, 62
sulfonamides and, III, 62
- Cinchol,
as growth factor for *Trichomonas columbae*, I, 261
- Cinobufagin,
crystal structure of, II, 441, 442, 445, 446
empirical formula, II, 445
molecular weight, II, 445
- Cinobufagone,
crystal structure of, II, 442
- Citric acid,
effect on choline acetylase, III, 367
- Citrin, see also Vitamin P
capillary resistance and, III, 4
interrelation with ascorbic acid, III, 4
- Climacteric,
gonadotropins and, V, 398, 408
male, V, 408
testosterone therapy in, V, 419
urinary gonadotropin titers in, V, 408, 409
- Clitoris,
effect of androgens on, V, 335
- Clostridia,
p-aminobenzoic acid requirement of, III, 173
biotin requirement, III, 169
inhibition by antibiotin factor, III, 169
oleic acid requirement, III, 213
uracil requirement, III, 199
- Clostridium butylicum*,
vitamin synthesis by, III, 28-30
- Clostridium tetani*, folic acid and, IV, 5
- Clupanodonic acid, II, 20
- Coccarboxylase, I, 171; III, 129
absorption spectrum of, I, 138
assay of, I, 149
effect on heart, V, 113
of vitamins on, II, 317, 337
intestinal absorption, III, 38
lactic acid and, IV, 88
pyrophosphoric acid and, III, 6
pyruvic acid and, IV, 88; V, 115
sources of, II, 16, 335; III, 6; IV, 86
thiamine and, III, 6; IV, 86
- Cod liver oil, III, 58, 77, 82
destruction of tocopherol by, III, 12
effect on tumors, II, 334
therapeutic value of, II, 19, 156, 322
toxic effects of, III, 12, 16
vitamin A activity of, II, 156, 167, 168
- Coenzyme I (diphosphopyridine nucleotide), III, 7, 180 ff.
absorption spectrum of, I, 137, 139
activity of derivatives of, III, 188
antagonism to sulfapyridine, III, 196
bacterial synthesis of, III, 188, 190
blood and, IV, 98
effect on tumors, II, 319, 320
functions of, I, 170, 171
identity with "V" factor, III, 186
in kidney, I, 242
in liver, II, 320, 335, 336
pellagra and, IV, 98
vitamins and, II, 319-339
- Coenzyme II (triphosphopyridine nucleotide), III, 180 ff.
absorption spectrum of, I, 137
bacterial synthesis of, III, 190
effect on tumors, II, 319
functions of, I, 171
identity with "V" factor, III, 186
in kidney, I, 242
niacin and, II, 319; IV, 95
Proteus vulgaris and, III, 192
- Coenzyme R, see also Biotin, III, 159, 160, 164, 165
bacterial synthesis of, III, 164, 165
requirement of *Rhizobia*, III, 159, 163-165
source of, II, 30
vitamins and, II, 31, 313
- Coenzymes,
absorption spectrum of, I, 139
catalytic function of, I, 170
determination of, I, 139
enzyme systems and, I, 264
niacinamide in, III, 7, 190, 193
relation to p-aminobenzoic acid, II, 233
to vitamins B, I, 137
- Cold,
corticotropic hormone and resistance to, V, 225
- Collagen,
ascorbic acid and, I, 168-170, 186, 242; IV, 100

- Colon,
 bacterial synthesis of riboflavin in, III, 28
 of thiamine in, III, 38
- Colon bacilli,
 effect on chicks in sterile environment, III, 24
- Colostrum,
 immune bodies in, IV, 40
 vitamins in, II, 117, 194; IV, 82, 96
- Concanavalin,
 p-aminobenzoic acid in, II, 239
- Conception,
 effect of protein on, III, 76
 of vitamin E deficiency on, III, 90
- Congenital defects,
 defective gene and, III, 97
 environmental disturbances and, III, 97
 maternal manganese deficiency and, III, 79
 vitamin deficiency and, III, 82, 83, 85, 88, 89, 97, 98
- Copper,
 achromotrichia and, II, 219
 inhibitory effect on choline acetylase, III, 366
 iron deficiency and, IV, 120
 in pregnancy diet, III, 94
- Coproporphyrin,
 pantothenic acid and secretion of, I, 176
 pernicious anemia and excretion of, I, 279
- Coprostanane,
 crystal structure of, II, 435, 447
- Coprostanone,
 metabolism, I, 322
- Coprosterol,
 crystal structure of, II, 447
 formation in animal organism, I, 322-324
- Corn,
 dental caries and, II, 268
 vitamin E in oil of, II, 115, 284
- Corpus luteum,
 androgens and formation of, V, 327
 extract,
 effect on the male mammary gland, V, 260
 isolation of
 allopregnanol-3(β)-one-20 from, I, 299
 progesterone from, I, 296, 297, 302, 303, 322
- Corticosterone, I, 361, 381
- Corticotropic hormone,
 analysis of, V, 220
 assay of, IV, 330; V, 215, 216
 biological properties, V, 221-226
 diabetogenic action, V, 224
 effect on carbohydrate metabolism, V, 224
 cartilages, V, 223
 hypophysectomized rats, V, 215
 liver arginase, V, 222
 lymphoid tissues, V, 225
 osseous system, V, 221
 seminal vesicle, V, 224
 serum proteins, V, 225
 growth hormone and, V, 221, 222
 isoelectric point of, V, 220
 isolation of, V, 216-219
 physical and chemical properties, V, 220, 221
 resistance to cold and, V, 225
 low atmospheric pressure, V, 225
- Cortilactin, in adrenal extracts, I, 369
- Corynebacterium diphtheriae*,
 growth factors for, III, 152, 154, 168, 173, 181, 212
 vitamin synthesis by, III, 136, 155
- Cotton seed oil,
 antagonism to cod-liver, III, 12
 preventive action in dystrophy, III, 12
 vitamin E in, III, 12
- Coumarans, antisterility
 activity of, II, 120
- Coumarins, antisterility
 activity of, II, 120
- Cozymase, see Coenzyme, I
- Creatine,
 arginase as precursor of, II, 146
 biosynthesis of, I, 25-27, 29, 179
 effect of steroid hormones on, IV, 279
 metabolism of, I, 157, 180
 nutritional muscle dystrophy and, 133, 146
 phosphorylation of, I, 186
- Creatinine, biosynthesis of, I, 26
 excretion of, I, 47, 179, 180
- Creatinuria,
 effect of androgens on, V, 379
 inositol on, III, 58
 steroid hormones and, IV, 280
- Cretinism, IV, 244
 iodine and sporadic, IV, 121
- Cretins,
 athyroid, sporadic, III, 80
 endemic,
 born of goitrous mothers, III, 80
 enlarged thyroid in, III, 80

- Crocetin, I, 210
 Crocin, I, 210
 Crop gland,
 effect of simultaneous administration
 of prolactin and estrone on, II, 392
 Cryptorchidism, V, 417, 418
 due to estrogen injection, V, 243
 treatment of, V, 418
 Cushing's syndrome,
 creatinuria in, IV, 286
 effect of androgens on, IV, 266, 271-
 274, 294; V, 374-376
 of estradiol on, IV, 276; V, 375
 Cyanides,
 inhibitory action on thyroxine forma-
 tion, V, 289
 β -Cyclocitral,
 syntheses of vitamin A from, II, 181
 Cystathionine,
 as growth factor, I, 30
 Cysteine,
 antilipotropic effect of, I, 19
 biological formation of, I, 30
 complex with dehydroascorbic acid,
 I, 184
 effect on enzymes, III, 367; V, 291
 of goitrogenous agents on, V, 291
 iodine reduction by, V, 293
 Cystine, I, 27-29, 33-39
 as amphicarcinogen, II, 344
 antilipotropic activity of, I, 23, 47, 50,
 181
 biotin and, II, 37
 in diet, I, 19-24, 27-29, 33-39, 46, 180,
 182; II, 20
 tumors and, II, 334, 340
 Cytochrome,
 occurrence, I, 262; II, 338
 Cytochrome C,
 in hepatomas, II, 338
 riboflavin in, II, 315
 Cytochrome oxydase, I, 262
 in hepatomas, II, 338
 inactivation of, II, 339
 Cytochrome reductase, I, 166
 Cytosine, absorption spectrum of, I, 128,
 129
 Cytopenia,
 prevention of, II, 96
 vitamins and, II, 91-100; III, 61
- D**
- Dark adaptation,
 effect of eggs on, III, 4, 5
 of halibut liver oil on, III, 4, 5
 of oranges on, III, 5
 vitamins and, III, 4-6, 17; IV, 84
 Dehydroandrosterone, II, 362
 activity of, II, 381
 effect on birds, II, 366, 374
 metabolic effects of, IV, 274
 cis-Dehydroandrosterone, II, 364
 activity of, 380
 trans-Dehydroandrosterone, II, 363
 activity of, II, 380, 381
 effect on birds, II, 374, 376
 structure of, II, 364
 Dehydroascorbic acid, V, 43
 complexes of, I, 184
 degradation of, I, 178
 reduction of, I, 179
 7-Dehydrocholesterol (provitamin D₃),
 absorption spectrum of, I, 118, 134, 135
 assay of, I, 138
 Dehydrocorticosterone, I, 313, 362
 adrenosterone from, I, 314
 Dehydrogenase systems,
 vitamins in, III, 6
 Dehydrogenases, I, 171
 inhibition of, I, 325
 5-Dehydrohomovitamin A,
 absorption spectrum of, V, 9
 conversion into vitamin A ethyl ethers,
 V, 20
 ethyl ether of, V, 5
 Dehydroisoandrosterone (Δ^5 -androsten-
 3 β -ol-17-one),
 conversion to Δ^5 -androstene-3,17-diol,
 I, 325
 to 3-chloro-17-androstenone, I, 312
 formation from cholesterol, I, 336
 isolation from urine, I, 309, 311, 317
 testosterone and, I, 316, 317, 319
 Δ^4 -Dehydroisoandrosterone, I, 312
 7-Dehydrositosterol,
 absorption spectrum of, I, 134
 7-Dehydrostigmasterol,
 absorption spectrum of, I, 134
 5-Dehydrovitamin A,
 biological activity, V, 14
 ethers, V, 9-22
 synthesis, V, 9 ff.
 ultraviolet absorption spectra of, V, 20
 Dermatitis, III, 55
 biotin and, I, 41; II, 35; III, 54, 57
 effect of choline on, II, 22
 fatty acids and, II, 21
 niacin deficiency and, I, 89
 sulfonamides and, III, 54
 Desoxycholic acid,
 carotene absorption and, I, 162

- degradation, I, 373
- Desoxycorticosterone, I, 365, 366; IV, 336
effect on shock, I, 362
excretion of pregnanediol and, I, 300, 302
functions of, IV, 354
progestational activity of, I, 367
as standard of adrenal cortical activity, IV, 343, 349, 352
unit of, IV, 337, 340
use in adrenal cortical dysfunction, IV, 266, 271, 274
- Desthiobiotin,
biotin and, II, 55, 59, 62
from biotin methyl ester, II, 56
- Desthiodiaminocarboxylic acid, II, 56-59
- Development,
effect of diet on, III, 95
estrogens on, V, 242-248
riboflavin on, III, 97
- Dextrimaltose,
effect on intestinal synthesis of thiamine, III, 31, 36, 39
- Dextrin, effect on vitamin B synthesis, III, 31
- Dextrose,
intestinal thiamine synthesis and, III, 31, 39
tolerance curves, IV, 172
- Diabetes mellitus,
alloxan, IV, 202
growth hormone and, V, 213
anterior pituitary and, V, 213
avitaminosis B and, IV, 173
blood sugar levels in, IV, 191
cancer and, IV, 167
corticotrophic hormone and, V, 224, 225
effect of liver extracts on, IV, 174
thiouracil on, IV, 202
nitrogen retention and, V, 212
nutritional aspects of, IV, 172-173
phlorhizin, IV, 202
thyroid and, IV, 193-199
vitamin B therapy of, IV, 175, 176, 179
vitamin excretion in, I, 98
- Diabetes insipidus,
vitamin excretion in, I, 98
- Diaminocarboxylic acids,
biotin and, II, 46-48, 50-51, 53-54, 62
- Diaminotetrahydrothiophene,
activity of, II, 60
from biotin, II, 55
and phenanthrenequinone, II, 53
quinoxaline derivative of, II, 57
- Dianhydrogitoxigenin, II, 441
crystal structure of, II, 442, 444
- Dianol, III, 232
- Diarrhea,
in nutritional cytopenia, II, 97
in pernicious anemia, I, 280
response of chronic, to pteroylglutamic acid, V, 154
in sprue, V, 124
vitamin deficiency and, I, 89-91; II, 72-74; III, 81
- Diazomethane, II, 42-44
biotin and, II, 42
- Dibenzanthracene,
effect on hepatic vitamin stores, II, 310, 311
similarity between estrogens and, II, 358
- Dibenzoquinoxaline, II, 57
- Dibromocholesteryl bromide,
crystal structure of, II, 435
- Dibromocholesteryl chloride,
crystal structure of, II, 435, 451, 452
- 2,6-Dichlorophenolindophenol,
ascorbic acid assay with, II, 322
- Dicholesteryl ether,
crystal structure of, II, 435, 451
- Dienestrol, III, 233
- Diet,
adequacy of, III, 1
artificial,
infantile malnutrition and, IV, 122
coprophagy and purified, III, 24
effect on absorption of hormones, IV, 144
of vitamins, IV, 144
intestinal bacteria, III, 31
estrogens and, IV, 137, 138
infection and, IV, 36, 45, 50 ff.
lactation and, IV, 139
maternal, IV, 142, 143
abortion and, III, 92, 95; IV, 74
effect on offspring, III, 75, 92-95, 98; IV, 74
parturition and, IV, 74
pregnancy and, III, 92
reproduction and, III, 92; IV, 136
- Diethylstilbestrol, see also Stilbestrol, III, 233; IV, 138
activity of, II, 386, 389; V, 234
antagonism to testosterone, II, 403
effect on birds, II, 389, 391, 399
glycogen, IV, 192
structure of, II, 387
- Digitoxigenin, II, 441
chemical structure, II, 444

- crystal structure, II, 442
 Digoxigenin, II, 441
 chemical structure, II, 444
 crystal structure, II, 442, 443
 Dihydrocalciferol,
 crystal structure, II, 454, 455, 456
 Dihydrocrocin methyl ester,
 structure of, II, 174
 17-Dihydroequilenin,
 isolation from urine, I, 328
 22-Dihydroergosterol (provitamin D₄),
 absorption spectrum, I, 134
 as growth factor for *Trichomonas*
 columbae, I, 261
 β -Dihydrofucosterol,
 crystal structure of, II, 435, 454, 455
 Dihydroquinoxaline, II, 52-54
 Dihydrotestosterone, II, 362
 activity of, II, 380
 configuration and, II, 381
 structure of, II, 363
 Dihydrotheelin, see α -Estradiol,
 Δ^4 -Dihydrotigogenone,
 metabolism of, I, 323
 Dihydrovitamin, A, II, 180
 4,4'-Dihydroxy- α , β -diethylstilbene, see
 Diethylstilbestrol
 4,4'-Dihydroxy- γ , δ -diphenyl-n-hexane,
 see Hexestrol
 3(β),17(α)-Dihydroxyetioallocholan-
 ic acid, I, 380
 3(β),17(β)-Dihydroxyetioallocholan-
 ic acid, I, 380
 Diiodotyrosine, III, 64; IV, 209, 216, 220
 effect on thyroid, V, 276
 factors influencing formation of, V, 291
 formation of thyroxine from, IV, 234,
 235, 238
 11,12-Diketocholan-ic acid, I, 371
 p-Dimethylaminoazobenzene, see also
 Butter yellow
 effect on enzyme systems, II, 339
 metabolism of, II, 305, 338, 339
 tumors and, II, 318, 320-323, 326, 331-
 339
 p-Dimethylaminobenzaldehyde,
 determination of sulfonamides with,
 II, 240
 Dimethylaminovitamin A,
 conversion to vitamin A, V, 25-27
 Dimethyl-p-phenylenediamine,
 effect on enzymes, II, 339
 as metabolite of p-dimethylaminoazo-
 benzene, II, 338
 Diphenylethane,
 estrogenic activity of, III, 231
 Diphenylethylene, see Stilbene
 Diphenyl- α -naphthylcarbinol,
 estrogenic activity of, III, 231
 Diphosphopyridine nucleotide, see Coen-
 zyme I
 Diphtheria, ascorbic acid and, IV, 107
Diplococcus pneumoniae,
 action of sulfonamides on, III, 175, 177
 choline requirement of, III, 210, 211
 ethanolamine as growth factor for, III,
 210
 glutamine requirement of, III, 146
 inhibition by antibiotin factor, III, 170
 Dopa activity, of p-amino-benzoic acid,
 II, 231
 Dopa oxidase reaction, of hair, II, 219
 Drosophila,
 action of thiourea on, V, 283
 Duck,
 vitamin E requirement of, II, 143, 122
 Dyscrasia,
 folic acid and, III, 30, 54
 intestinal synthesis and, III, 34
 L. casei factor and, III, 34
 liver extracts and, III, 54
 niacin and, IV, 20
 sulfadiazine and, IV, 23
 Dysentery,
 agglutinin and, IV, 56
 nature of, V, 154
 vitamin deficiency and, in monkeys,
 IV, 56, 85
 Dysmenorrhea, V, 351-356
 pathophysiology of, V, 351
 therapy with androgens, V, 353
 Dystrophy, adiposogenital, V, 417

E

- Ears, accessory, vitamin A deficiency
 and, III, 82
 Ectoplacenta,
 effect of vitamin E deficiency on, III,
 91
 Edema,
 protein deficiency and nutritional, IV,
 76, 77
 resistance to viruses and, IV, 42
 vitamin deficiency and, IV, 19, 90, 142
 Egg white,
 achromotrichia and, II, 218
 antibiotin factor and, IV, 139
 anticarcinogenic effect of, II, 344
 avidin in, II, 34
 effect on resorption of gonadotropin,
 III, 323

- toxicity of, II, 30, 31, 63; III, 57
- Egg yolk,**
 vitamins in, II, 37, 38, 66, 116, 197
- Eggs, see also Egg white, Egg yolk**
 p-aminobenzoic acid in, II, 242, 243
 dark adaptation and, III, 5
 shell-less, produced by estrone injection, II, 390
 vitamins in, IV, 143
- Ejaculate,**
 effect of estrogens on, V, 240, 241
- Electric eel (*Electrophorus electricus*),**
 chemical and electrical energy in, III, 361 ff.
 electric organs of, III, 351, 354, 355, 361
- Electric organs, of fish, III, 351-355, 361 ff.**
- Electrolytes,**
 effect of sex hormones on excretion of, IV, 289 ff.
- Embryo, see also Fetus**
 biotin in tissues of, II, 36, 314
 effect of androsterone on, II, 365
 estrone on, II, 389
 maternal diet on, III, 74, 76
 maternal vitamin deficiency and, III, 80-90, 96-98
 organogenesis of, III, 98
- Enamel, of teeth,**
 caries and, II, 256, 281-285, 299
 chemical composition of, II, 282
 effect of microorganisms on, II, 270
 weak acids on, II, 283
- Encephalomalacia,**
 nutritional, II, 122, 123, 126, 137, 138, 139, 140
 symptoms of, II, 139, 143
- End plates,**
 choline esterase in, III, 343-345, 353
 in electric organs, III, 352, 353, 357
- Endocervicitis,**
 effect of androgens on, V, 350
- Endometriosis, V, 347-349**
 pathophysiology of, V, 347
 therapy of, V, 347 ff.
- Enzyme systems,**
 riboflavin in, III, 97, IV, 91
- Enzymes,**
 absorption spectra of, I, 115, 137, 138
 in biological oxidations, I, 166
 coenzymes and, I, 170, 185
 flavoprotein, I, 138, 166
 as genes, III, 97
 goitrogenic agents and, V, 290-293
 inactivation of, II, 215, 228, 229, 230, 239
 intestinal, I, 286
 in liver, II, 336
 oxidative, I, 166
 proteolytic, I, 165, 286
 respiratory, III, 351, 358
 tumors and, II, 327, 336
 vitamins and, I, 170, 235, 245; II, 319, 322, 345; III, 97
 yellow, I, 166
- Epiallopregnanolone,**
 androgenic activity of, I, 313
 isolation of, I, 299
 synthetic, I, 313
- Epicoprosterol,**
 isolation of, I, 323
- Epilepsy,**
 effect of glutamic acid on, III, 366
 vitamin B₆ deficiency on, III, 10
- Epipregnanolone, see Pregnanolone**
- Epithelioma,**
 chorionic hormone in, II, 357
 effect of estrone on formation of, II, 354
 of vitamin A on, II, 312
- Epithelium,**
 effect of estrogens on, V, 258
 vitamin deficiency on, II, 190, 199-309, 320, 330; IV, 84
- Equilenin, I, 335; III, 230**
 isolation of, I, 327, 333; II, 385
- Equilin, I, 335; III, 230**
 crystal structure of, II, 436, 438
 isolation of, I, 327; II, 385
- β -Ergostadienetriol,**
 crystal structure of, II, 435
- Ergostanol,**
 as growth factor for *Trichomonas columbae*, I, 261
- α -Ergosterol,**
 crystal structure of, II, 435, 436
- Egoosterol (provitamin D₂),**
 absorption spectrum of, I, 114, 134, 135, 138
 assay of, I, 138
 crystal structure of, II, 435, 436, 447, 450, 452, 454-457
 as growth factor for *Trichomonas columbae*, I, 261
 irradiation of, I, 125, 261
 therapeutic use of irradiated, II, 75, 77-78
- Ergosteryl acetate,**
 crystal structure of, II, 435, 451

- Ergotetraene**,
 crystal structure of, II, 452, 453
 empirical formula of, II, 453
 molecular weight, II, 453
- Erythrocytes**,
 ascorbic acid and, I, 174, 175
 effect of liver on, I, 272 ff.
 of *P. lophuræ* on, IV, 42
 of pteroylglutamic acid on, V, 144, 147-149
 iron deficiency and, IV, 120
 pernicious anemia and, I, 270, 271, 273, 274, 278, 279, 281, 287
 production of, III, 274-277, 290, 291
 regeneration of, V, 147
- Erythrophagocytosis**, due to ferric chloride administration, II, 138
- Erythropterine**,
 hematopoietic properties of, IV, 26
- Escherichia coli***,
 p-aminobenzoic acid and, II, 221, 244, 245
 diet and intestinal, III, 31
 effect of sulfonamides on, III, 175, 178-180
 inositol synthesis by, III, 52
 methionine requirement of, III, 178
 niacin requirement of, III, 181, 190
 resistance to pyridoxamine, III, 130
 thiamine content of, III, 27
 vitamin synthesis by, III, 28-30, 39, 40, 53
- Eserine**,
 effect on acetylcholine liberation, III, 371, 372
 on acetylcholine synthesis, III, 366
 on electric organs, III, 355
 on ganglion stimulation, III, 369, 370
 on vagus stimulation, III, 338
 and permeability of cell membrane, III, 372
- Estradiol**, II, 387; III, 230
 absorption spectrum of, I, 119, 137
 action on cat, V, 258
 effect on creatinuria, IV, 287
 on Cushing's syndrome, V, 375
 on electrolyte retention, IV, 289-292
 on fecal nitrogen, IV, 287
 inactivation of, I, 325; IV, 138, 139
 occurrence, II, 385
 preparation from estrone, II, 385
 structural similarity to carcinogens, II, 358
 therapeutic use in adrenal cortical dysfunction, IV, 276
 in hypogonadism, IV, 138
 vitamins and, IV, 138
- α -Estradiol (dihydrotheelin)**, I, 329
 benzoate, activity of, V, 234
 choline and, IV, 138
 comparison with β -estradiol, II, 385
 diet and, IV, 138
 estrone and, I, 326, 331-333, II, 386
 isolation of, I, 327
 metabolism of, I, 330
 occurrence, II, 385
 oxidation by laccase, I, 324
 vitamins and, IV, 138
- β -Estradiol**,
 estrone and, I, 331, 332; II, 386
 formation of, I, 326
 isolation of, I, 328, 331
 metabolism of, I, 331
 occurrence of, II, 385
- Estradiol benzoate**,
 antagonism to androsterone, II, 403
 to testosterone, II, 403
 effect on birds, II, 389, 390, 392-394, 397, 400, 401
 estrone and, II, 389
- Estradiol dibenzoate**, II, 387
 effect on birds, II, 394, 395, 400, 401
 estrone and, II, 386
 isolation of, II, 385
- Estranediol**,
 isolation of, I, 335
- Estratriene**, II, 387
- Estratriene-3,17-diol**, see Estradiol
- $\Delta^{1,3,5}$ -Estratrien-3-ol-17-one**, see Estrone
- Estratriene-3,16,17-triol**, see Estriol
- $\Delta^{5,7,9}$ -Estratrien-3-ol-17-one**, isolation of, I, 335
- Estrin**,
 tumors produced by, II, 311
- Estrinase**, I, 325
- Estriol (theelin)**, II, 387; III, 230
 absorption spectrum of, I, 119, 137
 activity of, II, 386; V, 234
 antagonism to androsterone, II, 403
 conversion of estradiol to, I, 330, 333
 of estrone to, I, 331, 332
 crystal structure of, II, 436-438, 440
 dehydrogenation of, I, 334
 estrone and, II, 386
 isolation of, I, 318, 328, 329, 332; II, 385
 metabolism of, I, 329, 331
 oxidation by enzymes, I, 324; II, 229
 recovery of, I, 329
- Estrogen(s)**, see also under name of individual compounds, II, 387

- in abortion, I, 304
 - activity of, II, 386; V, 251 ff.
 - chemical structure and, II, 386
 - androgens and, II, 403; IV, 151, 161, 166; V, 235, 241, 261, 266, 267
 - assay of, II, 386, 400
 - chemical structure of, V, 234
 - dosage levels of, V, 261, 262
 - effect on accessory sex glands, V, 251-262
 - on acromegaly, V, 376, 377
 - on adrenals, V, 262, 263
 - on adult males, V, 236-241
 - on birds, II, 385-403
 - on body growth, V, 247
 - on castrated mice and rats, V, 255
 - epithelia, V, 258
 - on external genitalia, V, 238
 - on immature males, V, 241-248
 - on internal genitalia, IV, 138; V, 239, 240
 - on liver, IV, 138, 154
 - on postnatal development, V, 242-248
 - on skin, 238
 - endogenous, V, 234, 235
 - estrus cycle and, IV, 144
 - excess, manifestations of, IV, 144 ff., 169
 - thyroid therapy of, IV, 169
 - excretion of, I, 328, 329; V, 235
 - formation of, I, 337; III, 317 ff.
 - hypophysectomy and, V, 246
 - inactivation of, I, 317, 325, 334; IV, 148, 149
 - international standards, IV, 313
 - isolation of, I, 326-328
 - lactation and, IV, 141
 - metabolism of, I, 295, 330, 331
 - estrone and, IV, 139
 - thyroid and, IV, 139
 - occurrence, IV, 137; V, 235
 - oxidation by enzymes, I, 324
 - protection of males from effects of, V, 263-266
 - recovery of, I, 334
 - reduction of, I, 335
 - synthesis of, I, 317, 328
 - synthetic, II, 385
 - tumors and, II, 354, 357, 359
 - urinary, IV, 154
 - hypogonadism and, V, 396
 - vitamins and, IV, 137-140, 144, 149-162, 166
- Estrone (Oestrone, Theelin), II, 387; III, 230, 301, 317, 320
- absorption spectra of, I, 119, 137-139, 144
 - activity of, II, 386; V, 234
 - androgens and, II, 402, 403; V, 267
 - assay of, I, 138, 144, 151
 - cholesterol and, IV, 138
 - conversion to estradiol, I, 332; II, 385
 - to estriol, I, 332
 - to lactones, I, 315
 - crystal structure of, II, 435, 436, 438-440
 - effect on birds, II, 388, 389, 392-395, 398, 400, 401
 - on estrogen metabolism, IV, 139
 - estradiol and, II, 386, 389
 - estriol and, II, 386
 - formation of, I, 333; III, 317 ff.
 - inactivation of, I, 324
 - inhibition and, IV, 137, 138
 - international standard of, II, 386
 - isolation of, I, 318, 327, 330, 369; II, 385
 - liver and, IV, 137, 138
 - oxidation by tyrosinase, II, 228
 - prolactin and, II, 392
 - reduction of, I, 325
 - sensitivity of prostate to, II, 356
 - structural similarity to carcinogens, II, 358
 - tumors and, II, 354, 358
 - vitamins and, IV, 138, 139
- Estrone acetate, II, 400
- Estrone benzoate,
 - activity of, II, 400
 - effect on birds, II, 392
- Estrus,
 - effect of lysine on, III, 76
 - of phosphorus deficiency on, III, 77
 - of proteins on, III, 76
 - of sodium deficiency on, III, 78
 - of thallium salts on, III, 329
 - of underfeeding on, III, 74
 - of wheat on, III, 76
- estrogens and, IV, 144
- pituitary hormone and, IV, 136
 - vaginal, III, 76
 - vitamins and, III, 81, 84, 85, 90, 96; IV, 136, 139, 144
- Ethanolamine,
 - as growth factor for *Pneumococcus*, III, 210
- Ethinyl estradiol, activity of, II, 385
- Ethinyl testosterone, II, 364
- Ethionine, III, 178
- Ethyl androstanediol,
 - activity of, II, 380

- 17-Ethyltestosterone, IV, 273
- Ethyluracil,
therapeutic value of, V, 305
- 17-Ethynyltestosterone, metabolic effects of, IV, 273
- Etioallocholane, see Androstane
- Etioallocholanol, metabolism of, I, 326
- Etioallocholanedione,
metabolism of, I, 316, 318, 319
- Etioallocholan-3(α)-ol-17-one, see Androstosterone
- Etioallocholan-3(α)-ol-17-one, see Androstosterone
- Etioallocholan-3(β)-ol-17-one, see Isoandrosterone
- Etiocholane, I, 296, 319
- Etiocholanol,
isolation of, I, 311, 317, 319
- Etiocholanedione, I, 319
- Etiocholan-3(α)-ol-17-one,
isolation of, I, 310, 313-315, 317-319
- Euglenarhodon, I, 212
- Eunuchoidism,
gonadotropins and, V, 398
hypogonadotropic, V, 402, 407
- Extrinsic factor, I, 284-288; III, 242; IV, 19
folic acid and, IV, 19
nutritional macrocytic anemia, V, 139, 141
pernicious anemia and, I, 283; V, 53, 141
vitamin B complex and, IV, 1
- Eye(s),
androgenic activity and color of, II, 370
astaxanthin in, I, 223
carotenoids in, I, 166, 220
inositol deficiency and, I, 93
"spectacled," I, 93
vitamin deficiency and, I, 83, 88, 93; III, 8, 81, 82, 84, 97; IV, 85, 94
vitamins in, I, 215, 219, 223, 240; III, 5; IV, 92
- F**
- F₂ fraction,
urinary, and nicotinic acid deficiency, III, 29
- Factor GPF, *L. casei* factor and, III, 61
- Factor R, IV, 30
anemia and, IV, 17
choline and, IV, 17
egg hatchability and, IV, 16, 17
folic acid and, IV, 17
growth and, IV, 16-18
vitamin B₆ and, IV, 13
vitamin B₆ conjugate and, IV, 18
- Factor S, IV, 19, 30
anti-anemia activity and, IV, 19
egg hatchability and, IV, 16, 17
folic acid and, IV, 17
growth and, IV, 16-18
vitamin B₆ and, IV, 13
- Factor SLR,
activity of, III, 61
folic acid and, III, 61
- Factor "TS,"
as growth factor for *Trypanosoma cruzi*, I, 263
- Factor U, III, 60 ff.; IV, 18; V, 120
- Factor W,
lactation and, IV, 140
- Fallopian tubes,
effects of androgens on, V, 331
- Fasting,
effect on bone, III, 75
on cartilage, III, 75
on premature delivery, III, 74
- Fat,
accumulation in liver, II, 12
bacterial synthesis of, III, 23, 44
body, I, 241
vitamins and, I, 32, 241
choline and liver, I, 18 ff.
coefficient of digestibility of, I, 162
deficiency syndrome,
arachidonic acid and, III, 76
linolenic acid and, III, 76
deposition of, I, 165, 240
androgens and, V, 267
estrogens and, V, 267
dietary, I, 60, 69, 161, 164
availability of vitamins and, V, 58
fetus and, III, 76
effect on intestinal thiamine synthesis, III, 32
fecal, in sprue, V, 132, 136
metabolism, I, 45, 158; II, 3, 11, 24, 29-36, 140, 141
vitamins and, II, 13, 19, 29-36, 147
oxidation of biotin by rancid, III, 3
pregnancy and, III, 92
synthesis, I, 32
from carbohydrates, II, 3-7
from proteins, II, 7-11
tocopherol as antioxidant for, II, 123, 143; III, 3
transport of, I, 181
vitamin A and, IV, 79
vitamin content of, II, 117, 196, 309

- vitamin requirements and, III, 1, 11, 12, 18
 vitamin-sparing action of, III, 11, 12, 18
Fatty acids,
 antagonism to riboflavin, III, 11, 12, 17
 antirachitic effect of, III, 11
 biological synthesis of, II, 4, 7, 14-17
 deficiency
 skin lesions and, III, 11
 essential, II, 1, 4, 19-23
 vitamins B and synthesis of, II, 21-23
 hepatic, structure of, II, 20
 toxic effects of, II, 141; III, 16
 unsaturated, II, 19-23
 in cod liver oil, II, 13
 role in exudative diathesis, II, 139
 vitamin B₆ and metabolism of, III, 11; V, 73
Feathers,
 effect of androgens on, II, 373
 of estrogens on, II, 395, 397, 398, 401, 402
 of factor vitamin B₁₀ on, II, 223
 thyroid deficiency and, II, 373
Feces,
 feeding and weight of, III, 24
 growth factor and, III, 24-26
 vitamins and, III, 27, 28, 30, 32, 35-38, 39, 41, 50; IV, 86, 100
Fermentation factor, V, 121, 138
Fermentation *L. casei* factor, V, 57
 response to, in pernicious anemia, V, 151
Ferrous sulfate,
 effect on resorption of gonadotropin, III, 323
Fertility, IV, 135
 calcium deficiency and, III, 77
 dose (M.F.D.) of tocopherol, II, 118
 effect of inositol on, II, 223
 of testosterone on, II, 370; V, 416
 phosphorus deficiency and, III, 76
 proteins and, III, 76
 vitamin E and, II, 125, 136
Fetus,
 amino acids and, IV, 76
 bone development in, IV, 107
 effect of maternal diet on, III, 74, 75, 80, 91
 vitamin deficiency on, III, 81, 89
 resorption of, III, 113, 141
 vitamin deficiency and, III, 74, 77, 81, 84, 85, 95; V, 190
 vitamins and, III, 98; IV, 81, 96, 101, 108
Fibrin,
 vitamin K and, IV, 115
Fibrinogen,
 vitamin K and, IV, 115
Filtrate factor, see Pantothenic acid
Fish,
 effect on vitamin B absorption, V, 65
 electric organ of, III, 351 ff., 361 ff.
 vitamin A in liver oil of, II, 161, 162, 167-172, 195
Flagellates,
 effect of thiamine on, III, 123, 126
Flavin-adenine-dinucleotide, I, 13, 166, 183
 absorption spectrum of, I, 138
Flavins,
 specificity for *Lactobacillus* spp., III, 136
Flavobacterium,
 vitamin B synthesis by, III, 51
Flavoprotein(s), I, 166
 enzymes, I, 138
 as glycine oxidase, III, 7
Flavorhodin, I, 196
Fluorides,
 effect on choline acetylase, III, 366
Fluorine,
 dental caries and, II, 285-296
 effect on enzymes, II, 290
 on pigmentation of teeth, III, 10
 occurrence, II, 285, 286, 290, 292, 296
 toxicity of, II, 296, 298
Folic acid, see also *L. casei* factor, Pteroyl-glutamic acid, IV, 4-6; V, 75 ff., 121
 absorption spectrum of, I, 116, 132
 achromotrichial action of, II, 35
 biological synthesis of, II, 321, 345, V, 72
 sulfonamides and, IV, 23
 cancer and, II, 321
 conjugates of, V, 75, 76
 in diet, II, 222
 effect on graying of fur, II, 221
 on ovary, IV, 137
 empirical formula, IV, 5
 estrogens and, IV, 137, 138, 144
 extrinsic factor and, IV, 19
 factor R and, IV, 7, 8, 17, 21
 factor S and, IV, 17
 growth activity of, IV, 4, 5
 hematopoiesis and, IV, 1, 2
 identity with vitamin B₉, II, 223
 inactivation of, IV, 5
 lactation and, IV, 141

- macrocytic anemia and, V, 150
 norite eluate factor vs., IV, 5
 occurrence, I, 233, 234, 236, 237, 239,
 243, 244; II, 23, 335, 336; IV, 24
 "potential," V, 155
 requirements, V, 168, 170, 171, 188
 therapeutic use in blood dyscrasias, V,
 298
 in sprue, V, 154
 vitamin M and, IV, 21
 vitamins B and, IV, 15, 16
 xanthopterin and, IV, 25, 27
- Follicle-stimulating hormone (FSH),
 see also Gonadotropin, III, 298, 325,
 328, 329
 action of, III, 320, 321
- Food,
 calcium deficiency and consumption
 of, III, 76
 effect of restriction of, on reproduction,
 III, 74; IV, 136, 137
- Formalin,
 effect on resorption of gonadotropin,
 III, 323
- Fowl,
 effect of goitrogenic agents on, V, 315
- Fractures,
 ascorbic acid and union of, IV, 106
 calcium deficiency and spontaneous,
 III, 77
- Fruits, biotin in, II, 33
- Fucoxanthin,
 absorption spectrum of, I, 202, 204,
 205
 growth activity of, II, 162
 occurrence, I, 196
 in photosynthesis, I, 202
- Fungi,
 biotin in, II, 33
 inositol requirements of, III, 207, 209
 oleic acid requirements of, III, 213
 vitamin requirements of, III, 33, 142-
 145, 158, 166, 202
- G**
- Galactose,
 intestinal absorption of, IV, 188
- Ganglia,
 choline esterase in, III, 344-346, 347,
 350
 effect of acetylcholine on, III, 369
 eserine on, III, 369, 370
- Gastric hormone I, 288
- Gastro-intestinal tract,
 pellagra and, IV, 97
 vitamins and cancer of, II, 305, 324,
 328, 330, 331
- Gelatin,
 effect on resorption of gonadotropin,
 III, 323
- Genes,
 congenital abnormality and defective,
 III, 97
 as enzymes, III, 97
- Genital tract,
 effect of estrogens on, V, 238-240
 inanition and, IV, 136
 malignancy of, V, 350
- Geronic acid, II, 187
- Gitoxigenin, II, 441
 crystal structure of, II, 442, 444, 445
- Glands, endocrine,
 effect of p-aminobenzoic acid on, II,
 225
 inanition and, IV, 136
 malignancy and, II, 353
 vitamins and, II, 108, 123, 325, 345
- Glaucoma piriformis*,
 reaction to thiamine, V, 94
- Gliadin,
 effect on estrus cycle, III, 76
- Globin, iodination of, IV, 211, 240
- Glucic acid (Reductone),
 as growth factor for flagellates, I, 260
- Glukoascorbic acid,
 effect on mice, V, 189
 as growth factor for flagellates, I, 260
- d-Glucohepto-ascorbic acid,
 as growth factor for flagellates, I, 260
- Glucosamine,
 in liver, III, 254, 286
- Glucose,
 effect on intestinal vitamin B synthesis,
 III, 31
- Glutamic acid, III, 149-151
 effect on acetylcholine synthesis, III,
 367
 on choline acetylase, III, 366, 367
 on epilepsy, III, 366
 on fatty livers, I, 19, 29
 enzymes and, I, 171
 requirement of *Streptococcus zymogenes*,
 III, 146
- Glutamine, III, 146 ff., 171; IV, 56
 as bacterial growth factor, III, 146-150
 peptides, III, 150
 structural specificity of, III, 150
- Glutathione, V, 63
 as antipernicious anemia substance,
 III, 284
 ascorbic acid and, I, 157, 175, 186

- comparison with thiouracil, V, 293
- decomposition of histidine and, I, 184
- dehydroascorbic acid and, I, 179, 184
- effect on succinoxidase, V, 291
- hemoglobin, I, 175
- reducing action of, I, 178; V, 293
- role in hemorrhagic degeneration of kidneys, I, 181
- tissue functions of, I, 158
- Glycerol,
 - effect on resorption of gonadotropin, III, 323
- Glycine,
 - bile acids and, I, 158, 162
 - in biogenesis of creatine, I, 179
 - deficiency,
 - choline and, I, 40
 - flavoprotein as oxidase for, III, 7
 - as growth factor for protozoa, I, 255
 - lipotropic activity of, I, 19, 29
 - metabolism of, I, 182
 - reduction of, I, 160
- Glycocholic acid,
 - carotene absorption and, I, 162
- Glycogen, II, 9, 19, 133
 - ascorbic acid and, I, 180
 - deposition in adrenalectomized rats, IV, 337-341, 353, 354
 - effect of adrenalin on, IV, 192
 - of insulin on, IV, 192
 - on resorption of gonadotropin, III, 323
 - storage in liver, I, 50, 347
 - thyroid and, IV, 190, 196
- Glycosuria,
 - corticotropic hormone and, V, 224, 225
 - thyroid and, IV, 189, 192, 193, 196, 199, 200, 203, 204
- Goiter,
 - congenital atrophy of glandular tissue in, III, 80
 - degeneration of thyroid in, III, 80
 - diet and, III, 79
 - effect on respiration, III, 80
 - endemic, III, 80
 - iodine and, IV, 121
- Goitrogenic agents,
 - action mechanism of, V, 287-299
 - activity of, in plants, V, 275
 - chemical nature of, V, 303-305
 - clinical application of, V, 305, 306
 - dietary effects on activity of, V, 300
 - direct effects on thyroid, V, 295
 - distribution in body, V, 301
 - effect on adrenal glands, V, 295
 - amphibians, V, 281, 282, 286, 315
 - basal metabolism, V, 277
 - birds, V, 280, 281, 285
 - body growth, V, 283-287
 - bone marrow, V, 298
 - cytochrome oxidase, V, 291
 - fishes, V, 282, 286
 - fowls, V, 315
 - invertebrates, V, 283
 - iodine metabolism, V, 288
 - on ontogeny, V, 283
 - proteolytic enzymes, V, 292
 - reproductive organs, V, 296
 - reptiles, V, 281
 - temperature on activity of, V, 300
 - thyroid hormone synthesis, V, 293
 - thyroid substance on, V, 277
 - hypophysectomy and, V, 276, 294
 - mammary passage of, V, 283
 - metabolism of, V, 301-303
 - modes of administration, V, 300 ff.
 - organ lesions due to, V, 298
 - placental transmission, V, 283
 - plants containing, V, 276
 - relation between activity and oxidizability of, V, 293
 - reversibility of activity, V, 279
 - sex differences in response to, V, 299
 - species differences in resistance to, V, 301
 - thyroidectomy and, V, 285, 286
 - toxic reactions to, V, 306
- Gonadotropic hormones,
 - anestrus and, IV, 144
 - assay of, IV, 318-328
 - in castrates, V, 408
 - chorionic, III, 298 ff.
 - effect on creatinuria, IV, 283
 - excretion of, III, 304-307
 - fixation by placenta, III, 304, 312
 - therapeutic use in hypopituitarism, IV, 267
 - effect of androstanediol benzoate on, II, 369
 - estrogens and, V, 250, 251
 - excretion of, I, 302, 303
 - hypogonadism and, V, 397, 398
 - inactivation of, III, 298-300, 302, 303, 307, 309, 310, 312
 - international standard, IV, 323
 - lactation and, IV, 141
 - occurrence, III, 298, 301, 306, 307, 310, 323, 324, 327, 331; IV, 321, 322, 326
 - pituitary, III, 301 ff.
 - follicle-stimulating effect of, III, 314, 320, 323, 331

- hypophysectomy and, III, 323, 324
 resorption of, III, 323
 synergism between, IV, 322
 testicular tumors and, II, 353, 357
 vitamin B deficiency and, IV, 136, 137
 Gonadotropin, see Gonadotropic hormones
 Gonads,
 asymmetry of, in birds, II, 365, 368
 effect of corticotropic hormone on, V, 222, 223
 of estrone on, II, 392
 of hypertrophy of, on birds, II, 368
 of removal of, on birds, II, 373
 food restriction and function of, IV, 136, 137
 vitamin B complex and function of, IV, 137-140
Gongylonema neoplasticum (*Spiroptera neoplasticum*),
 squamous cell carcinomas and, II, 330
 Graafian follicle,
 effect of androgens on, V, 328
 Granulocytes,
 effect of succinylsulfathiazole on, IV, 23
 Granulocytopenia, III, 55
 caused by thiouracil, V, 298
 folic acid and, III, 30
 L. casei factor and, III, 53, 67; IV, 23
 sulfonamides and, III, 54, 62; IV, 23
 therapeutic use of vitamin B₁₂ in, III, 62
 vitamin B₁₂ and, IV, 23
 Graves' disease,
 effect of androgens on, V, 378
 therapeutic value of goitrogenic agents in, V, 305
 Growth, III, 55; IV, 73, V, 200, 208
 p-aminobenzoic acid and, IV, 140
 calcium and, III, 76, 77
 diet and, III, 95
 effect of carotenoids on, II, 161
 of cod liver oil on, II, 156
 of estrogens on, V, 243, 246
 of factor R, S and U on, IV, 17, 18
 hormones on, II, 359
 hypophysectomy and rate of, V, 200, 246
 inhibitory effect of corticotropic hormone on, V, 221, 222
 naphthoquinones on, III, 215
 inositol and, IV, 140
 intestinal bacteria and, III, 24
 vitamins and, II, 161, 189, 191, 198; III, 17, 24, 85, 97
 xanthopterin and, IV, 27
 Growth factors, I, 262, 265
 interrelations of, III, 111, 112, 140, 161, 165, 205, 206
 storage in bacteria, III, 139
 Growth hormone, of anterior pituitary, V, 198 ff.
 assay of, IV, 328-330; V, 198-215
 biological properties, V, 208-215
 continuous growth and, V, 200, 208
 corticotropic hormone and, V, 221, 222
 diffusion of, V, 205
 effect on chondrogenesis, V, 213
 osteogenesis, V, 213
 of pH on activity of, V, 207
 electrophoresis of, V, 233
 fractionation of, V, 203
 history of, V, 201, 202
 isoelectric point of, V, 203, 206
 isolation of, V, 201-206
 nitrogen retention and, V, 211, 212
 physical and chemical properties, V, 206-208
 Guanidine carbonate,
 goitrogenic activity of, V, 277
 Guanidoacetic acid,
 in creatine biogenesis, I, 179
 lipotropic factors and, I, 41, 47
 Guanine,
 antagonism to benzimidazole, III, 203
 as bacterial growth factor, III, 199-201
 Guanopterine,
 hematopoietic properties of, IV, 26
 Guanosine,
 as accessory factor to antipernicious anemia substance, III, 265, 266
 Guinea pigs,
 ascorbic acid requirement of, III, 89
 Gums,
 effect on resorption of gonadotropin, III, 323
 Guvacin, I, 160
 Gynecomastia, IV, 148, V, 407
 in cirrhosis of the liver, V, 154, 165
- ## H
- Hair,
 color of,
 calcium pantothenate and, II, 223
 peripheral nervous system and, II, 224
 vitamin B complex and, II, 224
 effect of egg white diet on, II, 30
 of estrogens on growth of, V, 238
 of iodine deficiency on, III, 79

- gray, p-aminobenzoic acid and, II, 215
estrogens and, II, 219
folic acid and, III, 54
melanin pigment in, II, 217
pantothenic acid deficiency and, III, 54; V, 183
sulfaguanidine and, III, 54
testosterone and, II, 219
vitamin D and, IV, 41
- Halibut liver oil,
effect on blood vitamin A levels, III, 4-5
on dark adaptation, III, 4-5
in maternal diet, III, 94
as source of vitamin A, II, 167, 168, 170, 171, 181
- Hay fever,
ascorbic acid and, I, 174
vitamin D and, I, 174
- Heart,
p-aminobenzoic acid in, II, 241
effect of goitrogenic agents on, V, 285
iodine deficiency and, III, 79
poisons, II, 441-447
sulfonamides and, III, 63, 67, 68
thiamine deficiency and, III, 84; IV, 88
vitamin content of, I, 236, 237, 240, 244; II, 117, 319; IV, 93
- Hematin,
as growth factor for protozoa, I, 259-262, 265
- Hematochrome, I, 212
- Hematopoiesis, I, 174, 287
isoquinine and, IV, 26
pterines and, IV, 25, 26
tyrosine and, IV, 26
vitamins and, IV, 4, 5-11, 15, 19
- Hemoglobin, IV, 120
degradation products of, I, 279
effect on resorption of gonadotropin, III, 323
fraction R and, IV, 17
as growth factor for flagellates, I, 261
iron and, I, 64; IV, 119, 120
pernicious anemia and, I, 270, 279, 287
vitamins and metabolism of, I, 94, 174, 186
- Hemolysin,
vitamins and production of, IV, 63
- Hemosiderin, II, 136
- Hemophilus bacteria, growth factors for, III, 110, 186 ff.
- Hemorrhage, III, 55
butter yellow and, I, 40
choline and, I, 29, 33, 34, 46, 181
cystine and, I, 181
methionine and, I, 46, 181
sulfonamides and, III, 54
vitamin deficiency and, I, 66, 94, 95, 102, 104, 176; III, 21, 67, 84, 91; IV, 116-118
- Heparin,
as antipernicious anemia substance, III, 288
- Hepatomas, see also Liver, tumors
choline and, II, 307, 321
coenzyme in, II, 320
cytochrome content of, II, 338
effect of p-aminobenzoic acid on, II, 321
enzymes and, II, 338, 340
formation of cystine in, II, 340
induction by azodyes, II, 307
p-dimethylaminoazobenzene, II, 315, 320, 326, 340, 343
inositol in, II, 321
protein in, II, 308
vitamins and, II, 305, 308, 313, 316, 318, 322, 326, 328, 337, 343
- Hernia,
arising from estrogens, V, 236
- Hesperidin,
effect on ascorbic acid requirement, III, 4
- Hexestrol, II, 358, 386; III, 233
activity of, V, 234
of derivatives of, III, 233
effect on carcinoma of breast, II, 357
on prostate, II, 356
properties of, II, 356, 387
- Hexuronic acid, see Ascorbic acid
- Hippulin,
isolation of, I, 327
- Histamine,
ascorbic acid, and, I, 184, 186
- Histidase, I, 183
- Histidine,
as accessory factor to antipernicious anemia substance, III, 279 ff.
ascorbic acid and, I, 176, 183, 184, 186
combination with iodine, IV, 210, 211
- Homocysteine,
metabolism of, I, 24, 25, 30, 180, 186
- Homocystine,
in diet, I, 24, 25, 27, 34, 50
growth of rats and, I, 50, 80
hemorrhagic kidney degeneration and, I, 181
liver fat, I, 19, 46
- Homogentisic acid,
excretion of, I, 184, 185, 187
- Homosexuality,

- androgens and, V, 418-422
- hormonal imbalance and, V, 421
- Homothyroxine, IV, 212
- Homovitamin A ethyl ether, V, 8
 - absorption spectrum of, V, 9
- Hormoner, II, 341
- Hormones, see also under name of individual hormones
 - absorption spectra of, I, 109, 115, 137
 - achromotrichia and, II, 219
 - of adrenal cortex, I, 345-413
 - amino-acidogenic, I, 176
 - p-aminobenzoic acid and, II, 215, 228
 - assay of,
 - by absorption spectrophotometry, I, 110, 137, 138, 151
 - by colorimetry, I, 142-144, 151
 - by fluorescence spectroscopy, I, 112
 - biosynthesis of, I, 337
 - effect on birds, II, 361-408
 - of diet on absorption of, IV, 144
 - interrelationship of vitamins and, IV, 137, 143, 144, 149 ff.
 - plant growth, I, 265
 - tumors and, II, 328, 353-359
 - vitamins and, II, 145, 327; IV, 136
- Hydrocarbons,
 - carcinogenic, I, 143; II, 324, 327
- Hydroquinones,
 - activity of, II, 120, 231
 - p-aminobenzoic acid and, II, 227
 - as antioxidant, III, 3
 - effect on growth response to vitamin A, III, 114
 - hair color, II, 220
 - tocopherols from, II, 144
- Hydroxychromans,
 - as antioxidants, III, 2
- 17-Hydroxycorticosterone, I, 356; IV, 336, 353
 - activity of, IV, 355-358
 - glycosuria and, V, 225
- 17-Hydroxydehydrocorticosterone, I, 357
- 11-Hydroxy-12-ketocholanic acid, I, 371
- Hydroxypantothenic acid, III, 156
- Hydroxyphenyllactic acid,
 - excretion of, I, 184, 185
- Hydroxyphenylpyruvic acid,
 - excretion of, I, 184, 185
- Hyperestrogenemia, premenstrual, V, 356
- Hyperkeratosis,
 - pellagra and, IV, 99
 - vitamin deficiency and, III, 11; IV, 82, 83
- Hyperthyroidism,
 - blood sugar level in, IV, 188, 190, 191
- diabetes and, IV, 189, 197-199
 - effect on absorption of galactose, IV, 180
 - on alloxan sensitivity, IV, 197
 - on insulin sensitivity, IV, 192
- pancreas and, IV, 196
- respiratory quotient in, IV, 191
- vitamin B complex and, I, 177; V, 64
- Hypertrichosis,
 - effect of androgens on, V, 335
- Hypervitaminosis A,
 - treatment with ascorbic acid, III, 16
 - with thiamine, III, 16
 - with vitamin D, III, 16
- Hypervitaminosis D,
 - in monkey, II, 75
 - treatment with vitamin A, III, 17
 - vitamin B complex, III, 17
- Hypochromia,
 - iron deficiency and, IV, 120
- Hypogonadism, V, 394-411
 - clinical features of, V, 400-406
 - definition of, V, 394
 - effect of estradiol on, IV, 276
 - of testosterone and its derivatives on, IV, 264, 271, 291
 - hypergonadotropic, V, 406, 408
 - hypogonadotropic, V, 401, 402
 - pathology of, V, 398-400
- Hypophysectomy, IV, 136
 - effect of estrogens following, V, 260, 261
 - on goitrogenic activity, V, 277
 - on ovulation, III, 313, 314
 - gonadotropin and, III, 323, 324, 327-329
 - growth hormone and, V, 208
- Hypophysis, see Pituitary
- Hypopituitarism,
 - effect of androgens on, IV, 267, 272-275
- Hypoprothrombinemia,
 - effect of *L. casei* factor on, III, 62
 - of pteroylglutamic acid on, V, 134
 - sulfonamides and, II, 220; III, 54, 56, 62
 - vitamin K and, III, 91; IV, 115-117
- Hypothyroidism,
 - blood sugar level in, IV, 191
 - in congenital goiter, III, 80
 - diabetes and, IV, 199-202
 - effect of p-aminobenzoic acid on, II, 230
 - glucose consumption in, IV, 191
 - glycogen in, IV, 191
 - insulin sensitivity and, IV, 192
 - iodine and, IV, 121

- Hypoxanthine**,
 absorption spectrum of, I, 129
 as bacterial growth factor, III, 200, 201
- I
- Immune bodies**,
 in colostrum, IV, 40
 placental transfer of, IV, 40
 in serum, IV, 40
- Impotence**, V, 418, 419
 in diabetes, IV, 163
 due to avitaminosis B, IV, 163
 to liver poisons, IV, 163, 165
 effect of testosterone, V, 419
- Inanition**, IV, 48, 49, 136
 effect of maternal, on fetus, III, 74, 75
 endocrine status and, IV, 136
 estrogens and, IV, 138
 genital tract and, IV, 136
 resistance to bacterial infection and, IV, 42-44, 48, 49
- Infant**,
 calcium and tetany in, IV, 113
 effect of maternal diet on, III, 75, 92-94; IV, 74
 nutritional anemia in, IV, 120-122
 plasma protein levels of, IV, 75, 76
 protein metabolism of premature, IV, 77
 vitamins and, III, 84; IV, 81, 82, 87-89, 92, 97, 102
- Infection**, resistance to,
 nutrition and, IV, 35-70
 vitamins and, I, 173; III, 81; IV, 50-57
- Inositol**, II, 23, 219, 223; IV, 53, 56; V, 74, 77 ff.
 p-aminobenzoic acid and, II, 220, 223, III, 33; V, 78
 in animal nutrition, III, 208
 as antialopecia factor, I, 93
 assay of, III, 208
 bacterial synthesis of, III, 23, 30, 35, 52, 69; V, 77
 biotin and, I, 245; II, 36
 choline and, I, 42
 combination with vitamin E, III, 68
 conversion to tetrahydroxyquinone, III, 209
 deficiency, IV, 64
 alopecia and, III, 30, 52, 63, 67
 effect on creatinuria, III, 58
 lactation, II, 233; IV, 140, 141
 muscular dystrophy, III, 58
 of vitamins B on synthesis of, III, 32, 35, 52
 fat synthesis and, II, 3, 6, 12
 formation of, II, 333
 as growth factor, II, 222; III, 207-209; IV, 139
 lipotropic effect of, I, 44
 occurrence, I, 229, 233, 234, 236-238, 244; II, 321, 335, 336; IV, 143
 phagocytosis and, IV, 64
 reproduction and, II, 223; IV, 139
 requirement of chick, V, 168
 of mice, V, 111
 respiratory activity of tissues and, I, 238
 role of, in exudative diathesis, II, 139
 "spectacled" eye and, I, 93
 tumors and, I, 39; II, 321
 vitamin M deficiency and, II, 91
- Insects**,
 nicotinic acid and, III, 123, 182
 nutrition of,
 biotin and, III, 166
 effect of symbionts on, III, 184
 pantothenic acid requirement of, III, 158
- Insulin**, II, 229
 concentration in pancreas, IV, 195
 effect on depancreatized dogs, I, 50
 liver glycogen, IV, 192
 metathyroid diabetes, IV, 193
 resistance to, IV, 172
 adrenalectomy and, I, 347
 secretion of, IV, 195
 sensitivity to, IV, 192, 201
- Intersexuality**, in birds,
 androgens and, II, 365, 366
 estrogens and, II, 389
- Intestinal tract**,
 scurvy and, IV, 105
 synthesis of thiamine in, IV, 86, 87
 vitamin K in, IV, 115, 116
- Intrinsic factor**, III, 242
 destruction by heat, I, 287
 interaction of extrinsic factor and, I, 285, 288
 pernicious anemia and, I, 283; V, 141
 proteolytic enzyme of gastric juice and, I, 286
- Invertebrates**,
 effect of goitrogenic agents on, V, 283
- Iodide**,
 effect on goitrogenic agents, V, 276
- Iodine**, IV, 121
 cretinism and, IV, 121

deficiency,
 dietary, and goiter, III, 79, 80
 effect of maternal, on offspring, III,
 79, 94
 symptoms of, III, 79, 80
 effect on pituitary, V, 279
 goiter and, III, 121
 goitrogenic activity and, V, 287-289
 metabolism, effect of goitrogenic agents
 on, V, 287-290
 radioactive, III, 64; V, 288, 289
 reaction with thioureas, V, 315
 thyroid and, IV, 121; V, 275, 279, 288,
 289
 thyroxine and, IV, 121
 xanthine oxidase and, V, 291

Iodinine,
 bacteriostatic activity of, III, 215

Iodocasein, IV, 209, 210, 213, 215, 217,
 222
 absorption spectrum of, IV, 240
 effect on body growth, IV, 245
 milk secretion, IV, 242
 hydrolysis of, IV, 225, 232
 thyroxine content of, IV, 225, 232

Iodogorgonic acid, IV, 209

β -Ionone, V, 2

β -Ionylideneacetaldehyde, II, 180; V, 1, 2

Iodopsin,
 dark adaptation and, II, 202
 occurrence, I, 223

Iodothyron, IV, 209

Iron,
 "active," I, 262
 amino acids and, I, 183
 ascorbic acid and, I, 171
 biological importance, IV, 119
 deficiency anemia, I, 59, 64; IV, 120,
 121
 in diet, I, 64, 70, 75-79, 82, 94; III, 80,
 92, 94, 95, 98
 requirements for, I, 69, 75, 76, 82
 sources, I, 175, 279; IV, 119, 120
 therapeutic use of, I, 64

Isoalloxazine derivatives,
 riboflavin activity of, III, 136

Isoamylamine,
 as toxic agent in cod liver oil, III, 16

Isoandrostanediol,
 formation, I, 319, 321

Isoandrosterone, II, 362, 363
 activity of, II, 380, 381
 crystal structure, II, 438, 440, 441
 formation of, I, 315, 318, 319
 isolation of, I, 308, 309, 317

α -Isoascorbic acid,

as growth factor for protozoa, I, 260

Isobutylthiouracil,
 therapeutic value of, V, 305

Isoguanine,
 hematopoietic properties of, IV, 26

Isonicotinic acid, III, 183
Proteus vulgaris and, III, 183, 184

Isosteres,
 of nicotinic acid, III, 183, 186
 of thiamine, III, 129

Isothiamine, I, 252; III, 125

Isothiourea derivatives,
 adrenaline and, V, 296

K

16-Ketoestrone, I, 334

α -Ketoglutaric acid,
 oxidation by enzymes, I, 171

17-Ketosteroids, urinary,
 effect of androgens on, V, 396
 hypogonadism and, V, 396

Ketotestosterone, II, 364

Ketotetrahydrophenanthrene,
 estrogenic activity of, III, 231

Kidney,
 ascorbic acid loss from, I, 241
 calcification of, II, 78
 choline and, I, 11, 34, 35, II, 338
 effect of goitrogenic agents on, V, 297
 steroid hormones on, IV, 298-300
 sulfonamides on, III, 55, 64-66
 vitamin deficiency on, II, 123; III,
 82
 enzymes in, I, 49, 166, 325; III, 6
 formation of calculi in, II, 202
 vitamin content of, I, 236, 237; II, 117,
 119; IV, 93

"Klinefelter's syndrome," V, 410, 427

Kryptoxanthin, II, 165

L

L_1 factor, IV, 140

L_2 factor, IV, 140

Labor,
 inadequate diet and complications of,
 IV, 74

Laccase,
 oxidation of estrogens by, I, 324

Lactation,
 p-aminobenzoic acid and, II, 215, IV,
 140, 141
 anterior pituitary and, IV, 141
 choline and, I, 42; IV, 140
 diet and, IV, 140

- effect of androgens on, V, 370
 - calcium/phosphorus ratio on, III, 78
 - estrogens on, IV, 141; V, 261
 - iodinated proteins on, IV, 241, 244
 - manganese deficiency on, III, 79
 - phosphorus deficiency on, III, 77
- factor W and, IV, 140
- gonadotropic factors and, IV, 141
- inositol and, II, 223; IV, 140, 141
- liver extract and, IV, 140
- malnutrition during, IV, 122
- ovary and, IV, 141; V, 259
- thyroid and, IV, 241
- vitamins and, I, 75, 82; II, 108; IV, 139-141
- yeast and, IV, 141
- Lactic acid,
 - formation of, in electric organs, III, 362
 - in nerve stimulation, III, 362
- Lactic acid bacteria,
 - adenine as stimulant for, III, 199, 200
 - p-aminobenzoic acid requirement of, III, 173
 - glutamine requirement of, III, 146, 148
 - guanine and, III, 199, 200
 - hypoxanthine and, III, 200
 - inhibitory effect of antibiotin factor on, III, 169
 - interrelations of growth factors for, III, 134, 140
 - uracil requirement of, III, 199
 - vitamins B and, III, 28, 118, 134, 144, 156, 181, 193
 - xanthine and, III, 200
- Lactobacillus acidophilus*,
 - dental caries and, II, 278
- Lactobacillus arabinosus*,
 - in biotin assay, II, 32
 - effect of p-aminobenzoic acid on, II, 239
- Lactobacillus bifidus*,
 - effect of diet on intestinal, III, 31
 - thiamine synthesis by, III, 28
- Lactobacillus casei*, II, 32, 37
 - biotin requirement of, III, 163
 - glutamine requirement of, III, 146, 149
 - granulocytopenia and, IV, 23
 - growth factors for, IV, 2-5, 10, 11, 18
 - inhibition by antibiotin factor, III, 169
 - response to pyridoxine, III, 139
 - riboflavin assay and, III, 137
- Lactobacillus casei* factor, see also Folic acid, III, 58, 61, 63; IV, 5-7, 23, 29; V, 120, 121
 - anemia and, III, 61, 62, 67
 - bacterial synthesis of, III, 53, 66
 - biotin and, III, 63
 - chromotrichia and, III, 62
 - deficiency, manifestations of, III, 61, 67
 - dyscrasia and, III, 54, 62
 - granulocytopenia and, III, 53, 67; IV, 23
 - hematopoiesis and, IV, 2
 - hemorrhagic anemia and, III, 62
 - lactation and, IV, 141
 - leukopenia and, III, 61, 62, 67; IV, 23
 - of liver, V, 121
 - porphyrin caking and, III, 57, 62, 67
 - properties of, IV, 6
 - relation to factor GPF, III, 61
 - factor U, III, 61
 - folic acid, III, 60, 61
 - vitamin B₆, III, 60, 61; IV, 6, 29
 - vitamin M, III, 61
 - S. lactis* R and, IV, 7
 - sulfonamides and, III, 56, 66, 68
 - urinary excretion of, in pernicious anemia, V, 151
- Lactobacillus delbrückii*,
 - folic acid and, III, 60; IV, 4, 5
 - norite eluate factor and growth of, IV, 3
- Lactobacillus spp.*,
 - inhibition of, III, 196, 205
 - pyridoxine and, III, 139
 - riboflavin assay and, III, 137
 - specificity of flavins for, III, 136
 - stimulating action of purines on, III, 199
 - of uracil on, III, 199
 - thiamine requirement of, III, 118
- Lactogenic factors,
 - lactation and, IV, 141
- Lactogenic hormone,
 - assay of, IV, 333
 - international standard, IV, 335
- Lactose,
 - intestinal synthesis of vitamins B and, III, 26, 31
- Lard,
 - effect on caries, II, 284
 - therapeutic value in fatty acid deficiency, II, 19
- Laurylhydroquinone,
 - effect on growth response to vitamin A, III, 14
- Lecithin, I, 15; II, 333
 - choline and, I, 13, 45-47, 165
 - effect on availability of vitamin A, V, 58

- as growth factor for *Neurospora*, III, 212
- phospholipides and, I, 28
- tumors and, I, 40
- Lemon juice,
 - effect on resorption of gonadotropin, III, 323
- Leucine,
 - ascorbic acid and, I, 183
 - dehydroascorbic acid and, I, 183
 - nonlipotropic activity of, I, 19
 - pernicious anemia and, I, 281
- Leucocytes,
 - ascorbic acid in, I, 66, 102
 - effect of concentrates of, on resorption of gonadotropin, III, 323
 - vitamin A deficiency on, II, 72, 74; III, 80-81
 - pernicious anemia and, I, 270, 271, 278
 - thiamine content of, II, 329
- Leuconostoc mesenteroides*,
 - pyridoxine synthesis by, III, 138, 139
 - riboflavin requirement of, III, 134
 - stimulation by uracil, III, 199
- Leucopenia, III, 55
 - anemia and, II, 92, 94
 - caused by thiouracil, V, 298
 - L. casei* factor and, III, 61, 62, 67; IV, 23
 - reticulocyte crisis in, II, 95
 - S. lactis* R factor and, IV, 7, 8, 30
 - in sprue, V, 124
 - sulfonamides and, III, 54, 62; IV, 22
 - vitamins and, II, 100, 102; III, 30; IV, 23; V, 216
 - xanthopterin and, IV, 26
- Leucophytes,
 - action of thiamine on, III, 123, 126
- Leucopterin,
 - hematopoietic properties of, IV, 26
- Leukemia,
 - ascorbic acid content of white cells in, II, 323
 - thiamine and, II, 318, 329
- Leydig cells, V, 404
 - failure of, V, 400
 - testosterone and failure of, V, 419
- Libido,
 - effect of androgens on, V, 362
 - pathophysiology of, V, 361
- Light,
 - effect on carotene synthesis, II, 159
 - comb response to androgens, II, 376
- ultraviolet,
 - absorption by thiamine, V, 103
 - effect on nerve excitability, V, 102
- vagus perfusion liquid, V, 112
- rickets and, II, 76
- tumors produced by, II, 325
- Linoleic acid,
 - antioxidant action of, III, 3
 - choline and, II, 21
 - essentiality of, II, 19, 20
 - fat deficiency syndrome and, III, 76
 - interrelations with vitamins, III, 2-3
 - in plant oils, II, 17
 - therapeutic value in dermatitis, II, 22
- Lipides,
 - essential fatty acid content of, II, 20
 - formation of, II, 1, 16, 132
 - occurrence, I, 307
 - synthesis of, II, 2
 - tocopherol and metabolism of, II, 148
- Lipocaic,
 - atherosclerosis and, I, 42
 - biotin and, II, 36
 - effect on liver, II, 13
 - lipotropic activity of, I, 32, 43, 44
 - role of, in exudative diathesis, II, 139
- Lipoxidase,
 - inhibition by tocopherol, III, 3
 - as oxidant for carotene, III, 3
- Liver,
 - active principle of, I, 287
 - amino acids and, I, 28, 36, 37
 - cholesterol in, I, 42; II, 13
 - choline and, I, 18, 25, 28, 31 ff., 42 ff., 50, 180, 181; IV, 137, 164
 - cirrhosis of, IV, 147, 153, 154
 - coenzyme I in, II, 320, 335, 336
 - diabetes and, IV, 176
 - effect of aminoazotoluene on, II, 310, 331, 341
 - dimethylaminoazobenzene on, II, 318, 332, 335, 337
 - effect on breeding, III, 6
 - carcinogenesis, I, 39
 - of goitrogenic agents on, V, 294
 - on malformations, III, 88
 - resorption of gonadotropin, III, 323
 - of sodium succinate on, II, 338
 - efficacy against anemias, I, 246
 - pernicious anemia, I, 97, 269, 270, 275 ff., 280 ff.
 - enzymes and, I, 166; II, 335; III, 6
 - lactation and, IV, 75
 - α -naphthylurea and glycogen of, V, 297
 - oxygen consumption of, II, 336
 - phospholipide metabolism in, I, 40, 50
 - plasma proteins and, IV, 75
 - poisons, IV, 153, 164-166
 - estrogens and, IV, 138

- fatty, I, 17, 18, 25, 28, 31 ff., 43 ff., 180;
 II, 6, 9, 11
 as growth factor for protozoa, I, 260,
 261, 263
 hematopoietic activity of, III, 240 ff.
 inositol in, II, 335, 336
 iron deficiency and, IV, 120
 as source of antipernicious anemia sub-
 stance, III, 237 ff.
 sulfonamides and, III, 54, 55, 63, 67, 68
 tumors of, see also Hepatomas, II, 312,
 315, 316, 331
 vitamins and, I, 86, 133, 176, 177, 183,
 186, 219, 221, 230, 231, 232, 235-
 237, 239, 240, 242-244, 246; II, 3,
 31, 33, 36, 42, 66, 117, 118, 197,
 309, 310, 315, 316, 318, 327, 335-
 337; III, 8, 59, 62; IV, 12, 51, 79-
 81, 91, 93, 101, 138, 160, 164
- Liver extract, III, 247
 administration of, I, 276-278
 allergy and, I, 282
 as antidote for sulfaguanidine, III,
 34, 53, 54
 deposition of cholesterol and, I, 44
 dyscrasia and, III, 54
 effect on glossitis, I, 283
 efficacy against anemias, I, 246
 pernicious anemia, I, 273, 276, 280,
 283; V, 142
 estrogen inactivation and, IV, 178
 hydrolysis of, I, 286
 macrocytic anemia and, IV, 90
 nonsaponifiable lipid fraction of, IV,
 178
 polycythemia and, I, 281
 reticulocyte response to, I, 276, 277
 therapeutic value in avitaminosis B,
 IV, 176, 177
- Liver fraction "G," I, 276, 286; III, 244,
 249, 251, 290
 relation to known vitamins, III, 245
- Lumichrome,
 conversion of riboflavin to, III, 3
- Lumiflavin,
 absorption spectrum of, I, 116
- Lumilactoflavin,
 absorption spectrum of, I, 130
- Lumisterol,
 absorption spectrum of, I, 134
 crystal structure of, II, 454, 455
- Lupus erythematosus,
 androgens and, V, 381
- Lutein,
 properties of, II, 165
 vitamin A activity of, II, 165
- Luteinizing hormone (LH), see Gonado-
 tropic hormones
- Lycopene,
 properties of, II, 165
 structure, I, 200, 218
 vitamin A and, II, 162, 165, 188
- Lymphoblastomata, II, 138
 effect of vitamin E on, II, 326
- Lymphocytes,
 corticotropic hormone and, V, 226
- Lymphopenia,
 corticotropic hormone and, V, 226
- Lysine,
 effect on edema, II, 89
 on estrus cycle, III, 76
- M**
- Macrocytosis,
 reduction after pteroylglutamic acid
 therapy, V, 149
- Magnesium deficiency,
 effect on teeth pigmentation, III, 10
- Malaria,
 p-aminobenzoic acid and, II, 236
- Males,
 sterility in, II, 107, 124
 susceptibility of, to muscular dys-
 trophy, II, 144
- Malformations,
 congenital, and postnatal behavior,
 III, 97
 resulting from maternal diet, III, 95
 effect of liver on, III, 88
- Malnutrition, I, 62, 104; IV, 49-62
 detection of, I, 97
 of infants on artificial diet, IV, 122
 lactation and, IV, 121
 ocular symptoms of, I, 88
 pregnancy and, IV, 122
 prevalence of, I, 60
 resistance to infection and, IV, 48-62
 vitamin availability and, V, 79
- Mammary gland, see also Breast
 action of androgens on, V, 364
 effect of corpus luteum extract on the
 male, V, 260
 estrogens on, V, 259-262
- Man,
 ascorbic acid requirement of, III, 89
- Manganese,
 in achromotrichia, II, 219
 deficiency, I, 40
 congenital debility and, III, 79
 effect on lactation, III, 79
 ovulation, III, 79

- sexual maturity, III, 79
 skeleton, III, 79
 in maternal diet, III, 94
 thyroid and, IV, 218, 238, 239
 Mapharsen, see Arsenicals
 Mastitis, cystic, IV, 152, 154, 160
 estrogen therapy of, IV, 161
 following thyroid therapy, IV, 170
 nutritional therapy of, IV, 166
 Mating,
 effect of calcium deficiency on, III, 76
 of phosphorus deficiency on, III, 78
 of sodium deficiency on, III, 78
 of vitamin A deficiency on, III, 81
 Melanin, II, 217, 218, 219, 232
 Melanoma,
 vitamin A content of, II, 309
 Meningococci,
 inhibitory action of sulfonamides, on,
 III, 177
 Menometrorrhagia, IV, 147, 160
 estrogen therapy of, IV, 161
 Menopausal syndrome,
 pathophysiology of, V, 358
 therapeutic effect of androgens on, V,
 358
 Menorrhagia, V, 359
 in cirrhosis of liver, IV, 153
 in liver poisoning, IV, 165
 in pellagra, IV, 147
 in scurvy, I, 95
 therapeutic effect of androgens, V, 343
 vitamin B complex, IV, 161
 vitamin K deficiency and, IV, 159
 Menstruation,
 diseases aggravated by, V, 380
 effect of androgens on, V, 333
 estrogen excretion during, I, 302, 303,
 328
 pathophysiology of, V, 339-341
 vitamin A deficiency and, II, 72, 74, 75
 Mercaptothiazoline,
 modes of administration, V, 301
 Metabolism,
 amino acid, I, 157, 158, 161
 animal, I, 157, 159
 bacterial, I, 232
 basal,
 effect of goitrogenic agents on, V,
 275, 284
 of steroid hormones on, IV, 169,
 292
 sulfonamides on, III, 63, 69
 thyrotropin on, V, 284
 thyroxine on, III, 64; V, 293
 calcium, I, 96
 carbohydrate, I, 158, 347, 348
 cellular, I, 241
 creatine, I, 157
 fat, I, 158
 growth-promoting extract and, V, 212
 hemoglobin, I, 174
 hormones and, I, 158, 293-343
 protein, I, 165
 relationship between proteins and
 vitamins, I, 157
 vitamin A and, II, 330
 vitamin E and, II, 124, 325
 Metamorphosis, amphibian,
 inhibitory effect of thiouracil on, V,
 282
 thiourea on, V, 282
 Methathyroid,
 diabetes and, IV, 193, 195
 Methemoglobin,
 vitamins and, I, 175
 Methionine,
 antagonism to sulfonamides, III, 178
 antihemorrhagic effect of, I, 33, 34
 bacterial synthesis of, III, 179
 choline and, I, 40, 41, 180, 181, 182, 186
 cirrhosis and, I, 36, 37
 in diet, I, 30, 46, 50, 180
 discovery of, III, 111
 effect on estrogen inactivation, IV, 150
 formation of, I, 51, 180, 186
 lipotropic effect of, I, 20 ft., 44
 nitrogen retention and, IV, 271, 272
 requirement of *Escherichia coli*, III, 178
 Methylaminoazobenzene,
 hepatomas induced by, II, 320
 2-Methyl-4-amino-5-hydroxymethyl-
 pyrimidine, I, 252
 2-Methyl-4-amino-5-thioformylamino-
 ethylpyrimidine, I, 252
 Methylandrostanediol,
 activity of, II, 380
 effect on creatinuria, IV, 284, 287
 metabolic effect of, IV, 274, 275, 287
 renotropic effect of, IV, 300
 Methylandrostenediol,
 activity of, II, 380
 Δ^5 -Methylandrostene-3(β),17-diol, I, 319
 Methylcholanthrene,
 effect of vitamins on, II, 316, 318
 on vitamin A, II, 310
 tumors induced by, II, 318, 321
 vitamin E and, II, 326
 β -Methylcholine, I, 41
 Methylcyanide,
 goitrogenic activity of, V, 276
 S-Methylcysteine, I, 30

- Methyldiethylcholine, I, 41
- Methyldihydrotestosterone, II, 365
- activity of, II, 380
- Methylglyoxal,
beriberi and, IV, 90
- thiamine deficiency and, IV, 88
- Methyl linoleate,
nutritional value of, II, 20
- N'-Methylniacinamide,
chloride of,
sulfonamides and excretion of, III, 41
- F₂ fraction as derivative of, III, 29
- 2-Methyl-3-phytyl-1,4-naphtoquinone,
see Vitamin K
- Methyltestosterone, I, 319
- clinical use of, V, 339-381
- creatinuria due to, IV, 284
- effect on urinary urea, IV, 278
- metabolic effects of, IV, 271-273
- modes of administration, V, 411-416
- relative activity, V, 319 ff., 339 ff.
- therapeutic value in hypogonadism, V, 411 ff.
- β -(4-Methyl-thiazolyl-5)-alanine, I, 159
- Methylthiouracil,
effect on basal metabolism, V, 284
- thyroid, V, 280 ff.
- toxicity of, V, 316
- Metrorrhagia, IV, 153, 167
- in cirrhosis of liver, IV, 153
- in liver poisoning, IV, 165
- Mice,
alopecia in, III, 207, 208
- effect of riboflavin on, V, 181
- relative growth rates of, V, 175, 176
- thiamine deficiency in, II, 132; V, 176, 177
- vitamin requirements of, V, 175-194
- Microcytosis,
iron deficiency and, IV, 120
- Microorganisms,
symbiosis and vitamin requirement of, III, 33
- synthesis of vitamins by, III, 23
- thiamine requirement of, III, 117 ff.
- Milk, p-aminobenzoic acid in, II, 242
- breast, beriberi in infants and, III, 84
- thiamine deficiency and, III, 84
- effect on resorption of gonadotropin, III, 323
- fluorine in, II, 286
- iron in, IV, 120
- rickets and, IV, 108, 109
- transmission of mammary cancer through, II, 358
- vitamin K and, IV, 116
- vitamins in, II, 31, 33, 41, 196-197; III, 27-29; IV, 24, 25, 81, 82, 87, 89, 91, 92, 96, 97, 108
- Mineral deficiency,
prenatal development and, III, 76-80
- Mineral oil,
effect on vitamin absorption, II, 204
- Miotin, II, 342
- Miscarriage,
maternal diet and, III, 92
- vitamin B deficiency and, III, 84
- Monkey,
calcium deficiency in, II, 77, 79
- deficiency diseases in, II, 74-75, 79, 86, 88
- hypervitaminosis in, II, 75
- tuberculosis in, II, 76
- vitamin requirements of, II, 33, 79; III, 89
- Monoiodoacetic acid,
as a nerve poison, V, 99
- thiamine accumulation in nerve and, V, 115
- Moulting,
effect of thiourea on, V, 282
- Mucous membranes,
vitamin deficiency and, I, 90, 94, 95, 175
- Mullerian tissue,
response to estrogens, V, 258
- Muscle,
p-aminobenzoic acid in, II, 244
- calcification of, III, 55, 67
- choline esterase in, III, 342, 346, 347
- dystrophy, I, 41, 93, 170, 179
- androgens and, V, 379, 380
- avitaminosis E and, V, 191
- creatine loss in, II, 133
- nutritional, II, 107-109, 120, 128-130, 131, 141, 142
- susceptibility of males to, II, 144
- estrogens and development of smooth, V, 251 ff.
- glycogen level in, II, 133
- lesions of, II, 123, 130, 131, 135
- metabolic function of, II, 133
- refractory period of, III, 342
- relation of striated, to electric organ, III, 352
- steroid hormones and, IV, 300, 301
- sulfonamides and, III, 54, 63, 67, 69
- vitamins and, I, 93, 94, 166, 170, 179, 236, 237; II, 100, 110, 117, 118, 123, 135, 136, 143, 199, 315; III, 10, 67, 69; IV, 92, 114; V, 152, 178, 179, 191

- Myobacteria**,
 growth factors for, III, 110, 213-215
 production of phthiocol by, III, 213-215
- Myoma**,
 effect of androgens on, V, 346
 pathophysiology of, V, 346
- Myometrium**,
 effect of androgens on, V, 330, 331
 functional uterine bleeding and, V, 341
- Myxedema**,
 in endemic cretinism, III, 80
 iodine deficiency and, III, 79
- N**
- Naphthoquinones**, III, 213-217
 reversal of growth inhibition by, III, 215
- α -Naphthylthiourea**,
 effect on liver glycogen, V, 297
- α -Naphthylurea**,
 effect on rats, V, 315
 dogs, V, 315
- "Necrosin," V, 341
- Nematospora gossypii***,
 inositol requirement of, III, 207
 vitamin requirements of, III, 166, 209
- Neocryptoxanthin A**,
 provitamin A activity of, V, 32
- Neovitamin A**, V, 32, 33
 biological potency of, V, 33
- Nephrectomy**,
 gonadotropin excretion and, III, 308
- Nephrosis**,
 use of testosterone propionate in, IV, 270
- Nerve(s)**,
 acetylcholine and, V, 94-96
 action potential of, III, 353, 359, 360
 beriberi and, III, 10
 chemical transmitter theory of, III, 340, 360
 choline esterase in, III, 342-345, 348 ff.
 effect of prostigmine on, III, 372
 of ultraviolet light on, V, 102
 vitamin deficiency on, III, 10, 82; V, 114
 electric theory of, III, 340, 360
 fibers,
 effect of electric excitation on living, V, 101
 fluorescence of single, V, 105
 ultraviolet absorption of, V, 99
 heat production of, III, 337, 365
 phosphate bond energy in, III, 363, 365
 poisonous effect of monoiodoacetic acid on, V, 99
 thiamine and, V, 96-111, 114
- Neurasthenia**,
 vitamin deficiency and, I, 73, 84, 85, 90, 91, 100
- Neurine**, I, 1, 3-7, 15
 detection of, I, 11, 15
- Neurospora**,
 choline requirement of, III, 212
 lecithin requirement of, III, 212
 uracil requirement of, III, 202
- Neurospora crassa***,
 p-aminobenzoic acid as growth factor for, II, 239, 241
- Neurospora crassa* mutants**, III, 142-145, 158, 173, 177, 202, 212
 p-aminobenzoic acid requirement, III, 173, 177
 choline requirement, III, 212
 panthothenic acid requirement, III, 158
 pyridoxine synthesis by, III, 143
 pyrimidine requirement, III, 202
- Neutropenia**, malignant, I, 281
- Niacin**, see also Nicotinic acid, III, 63; IV, 54, 57, 95-100
 absorption spectrum, I, 132, 137, 139
 analogues of, II, 342
 assay of, I, 144
 biosynthesis of, I, 160, 238; III, 23, 28, 29, 41, 42, 51, 58, V, 69-71
 blood coagulation and, I, 176
 coenzymes and, II, 17; IV, 96
 competitive substitutes for, II, 442
 congenital malformations and, IV, 142
 deficiency, II, 89, 90, 102; III, 28; IV, 85
 low protein diet and, I, 70
 manifestations of, I, 61, 88-92, 101, 175, 182
 trigonelline and, III, 11
 urinary F_2 fraction and, III, 29
 derivative in cereal products, V, 83
 distribution of, I, 79, 182, 233 ff., 242, 244, 283; III, 8; IV, 95, 96-98, 143
 effect on antibody production, I, 172
 on breeding, III, 87
 on choline deficiency, III, 8
 on protein appetite, I, 161
 excretion of, I, 165, III, 8, 28; IV, 96, 98
 as growth factor for protozoa, I, 258
 interrelationships with other vitamins, II, 91; III, 7, 8, 15, 28, 33

- metabolism of, I, 182
 in nervous disorders, I, 85-86, 92
 nutritional cytopenia and, II, 93, 96
 pellagra and, II, 89, 90
 requirements of man, I, 74, 82; IV, 97
 of animals, V, 167, 169-171, 189
 response of yeast to, V, 47
 tumors and, V, 316, 319, 320, 339
- Niacinamide (nicotinamide),
 absorption spectrum of, I, 116, 132
 assay of, I, 138
 as bacterial growth factor, III, 181-183, 186 ff.
 bacterial synthesis of, III, 4, 190, 191
 carbohydrate metabolism and, III, 6
 chemical synthesis of, III, 185
 coenzymes and, I, 170; III, 7, 190, 193
 in dehydrogenase systems, III, 6
 dysentery bacilli and, III, 191, 193
 in nucleotides, III, 7
 protozoa and, I, 258, 264
 tumors and, II, 319
- Nicotinic acid, see also Niacin
 activity of derivatives of, III, 183-193
 bacterial synthesis of, III, 190, 192
 betaine of, I, 29
 effect on bacteria, III, 165, 192-195
 interrelations with biotin and thiamine, III, 165, 166
 isosteres of, III, 183, 186
 pyrazine analogs of, III, 183, 186
 requirement of bacteria, III, 181-185, 187, 190, 194, 196
 insects, III, 182
 roots, III, 182, 183
 specificity of, III, 184 ff.
 yeasts and, III, 183
- Nicotinuric acid, I, 160, 182
 urinary excretion of, IV, 96, 98
- Night blindness,
 vitamin A deficiency and, I, 64, 195; II, 74, 200
- Nitriles,
 goitrogenic activity of, V, 275
- Nitrogen,
 effect of steroid hormones on fecal, V, 287, 288
 on nonprotein, IV, 277, 278
 growth hormone and, V, 211, 212
 retention,
 alloxan-diabetes and, V, 212
 effect of steroids on, IV, 259-277
- Norite eluate factor, III, 56; IV, 2, 3; V, 120
 bacterial growth and, IV, 3
 chick growth factor and, IV, 3
- folic acid and, III, 60; IV, 5
 sulfaguanidine and, IV, 24
 vitamin B₉ and, IV, 9, 10
- Nucleotides,
 niacinamide in, III, 7
 riboflavin in, III, 7
- Nutrition, I, 60-104
 antibody formation and, IV, 63 ff.
 bacterial vitamin synthesis and, III, 23, 42-43
 caries and, II, 267, 293
 deficient, manifestations of, III, 73-103; IV, 73-122
 inositol and, III, 208
 pregnancy and, I, 77
 resistance to infection and, IV, 41-65
 sulfanamides and, III, 49
 tumors and, II, 305, 306, 317, 318
 vitamins and, I, 159
- O
- Oestradiol, see Estradiol
 Oestriol, see Estriol
 Oestrone, see Estrone
- Oil,
 effect on carotene absorption, V, 59
 toxic effects of, III, 12, 16
- Oleic acid,
 as growth factor for bacteria, III, 212, 213
 for fungi, III, 213
- Oleyl alcohol, vitamin A activity of, II, 167, 311
- Orchiectomy,
 urinary gonadotropins and, V, 406
- Ornithine, I, 160
- Osseous system,
 effect of corticotropic hormone on, V, 221, 222
- Osteogenesis,
 growth hormone and, V, 200
- Osteomalacia,
 in monkey, II, 77, 78
 vitamin D and, I, 95
- Osteoporosis,
 postmenopausal, V, 376
 treatment with androgens, IV, 265, 274
 vitamin D and, I, 103
- Osteosclerosis,
 fluorine and, II, 294-295
- Otosclerosis,
 effect of androgens on, V, 381
- Ovarian hormones,
 response of feathers to, II, 395, 398

Ovariectomy,
 effect on birds, II, 366-368, 395
 excretion of androgens following, I, 308, 309, 310
 of estrogens following, I, 302, 329-331

Ovaries,
 androgen secretion by, I, 308
 dysfunction of, II, 127
 effect of androgens on, II, 368; V, 327
 estrogen isolation from, I, 326-328; II, 385
 lactation and, IV, 141
 nutrition and, IV, 136
 pituitary hormones and, IV, 136, 137
 vitamin B deficiency and, IV, 137
 vitamin content of, I, 237, 240

Oviduct,
 effect of androgens on, II, 368, 392
 of estrogens on, II, 389, 390, 392; IV, 137
 vitamin B deficiency and, IV, 137

Ovotestis,
 androgens and, II, 365
 estrogens and, II, 388, 390

Ovo-verdin, I, 213

Ovulation, III, 311, 313, 322, 331
 effect of hypophysectomy on, III, 313, 314
 dietary fat on, III, 76
 mineral deficiency on, III, 78, 79
 vitamin A deficiency on, III, 81
 protein and, III, 76

Ovum,
 development in mammals, III, 95
 inadequate nutrition and implantation of, III, 74

Oxidase,
 d-amino acid, I, 166
 choline, I, 49
 cytochrome, I, 262
 liver aldehyde, I, 166
 polyphenol, I, 325
 xanthine, I, 166

Oxidation-reduction potentials,
 in thyroid, V, 292

Oxidation-reduction reactions,
 ascorbic acid and, I, 241; II, 322
 carotene and, I, 197
 coenzymes and, I, 139, 185
 effect of p-phenylenediamine on, II, 338
 of succinic acid on, II, 338
 in plants, I, 198, 210, 242
 vitamin E and, II, 147
 vitamin K and, II, 327

Oxycarotenes,
 properties of, II, 165
 structure of, II, 164
 vitamin A activity of, II, 165

P

PABA, see p-Aminobenzoic acid

PMS, see Gonadotropic hormones

PU, see Gonadotropic hormones

Palmitic acid, II, 8, 14, 22, 434

Palmityl ascorbic acid,
 covitamin action with tocopherol, III, 14

Pancreas,
 ascorbic acid and, I, 241
 biotin in, II, 33
 effect of liver extract on disorders of, I, 283
 lipotropic activity of, I, 17, 32, 42 ff.
 vitamin A absence in, I, 240

Pancreatectomy,
 effect on diabetes, IV, 193, 194, 200, 202
 of thiouracil following, IV, 202
 of vitamins B following, IV, 175

Panhypopituitarism,
 gonadotropin and, V, 398

Pantoic lactone, III, 153, 155, 156
 requirement of hemolytic streptococci for, III, 155

Pantothenic acid (Pantothen), III, 63, 151 ff.
 absorption spectrum of, I, 132
 achromotrichia and, I, 93; II, 221, 223
 alopecia and, I, 93
 analogs of, II, 342; III, 156, 157
 lactic acid bacteria and, III, 156
 biosynthesis of, III, 23, 29, 32, 35, 58, 155; V, 72
 breeding and, III, 87
 congenital malformations and, IV, 142
 deficiency,
 estrogens and, IV, 138
 lactation and, IV, 141
 manifestations of, I, 242; III, 54, 57, 58, 67; V, 183
 resistance to bacterial infection and, IV, 44, 45, 49, 55
 distribution, I, 229, 234-237, 239, 243-245; II, 319, 335, 336; III, 29, 62; IV, 143
 effect on depigmentation, II, 218
 on enzymes, II, 319
 on fat synthesis, II, 3, 9, 17
 on liver fat, I, 31, 32

- on weight, III, 88; V, 182
- on yeast growth, II, 30
- graying of fur and, I, 93
- as growth factor for protozoa, I, 256, 258
- identity with chick-dermatitis factor, I, 93; III, 29
- inositol synthesis and, III, 52
- insect nutrition and, III, 158
- interrelationship with other vitamins, I, 244; III, 7, 33, 58, 63
- porphyrin production and, I, 176
- requirements of bacteria, III, 152-154, 158
 - of mice, V, 182
 - of poultry, V, 166, 167, 170, 171
- response of microorganisms to, V, 46
- storage in liver, I, 243
- structure of, III, 153
- sulfonamides and, III, 35, 54, 57
- therapeutic value in dermatitis, II, 22
- thyroid and requirement for, IV, 139
- tumors and, II, 316, 319
- vitamin M deficiency and, II, 91
- Pantoyltaurine, III, 156
 - effect on mice, V, 191
- Papain,
 - ascorbic acid and, I, 171
 - enzymatic release of B vitamins by, I, 234, 235
- Parathyroids,
 - and fetal skeleton calcification, III, 78
- Paresthesia,
 - in Addisonian anemia, I, 97
 - in biotin deficiency, I, 94
 - in malnutrition, I, 81
 - in thiamine deficiency, I, 85
 - in pernicious anemia, I, 270
- Parkinson's disease,
 - pyridoxine and, I, 92
- Parturition,
 - and vitamin B deficiency, III, 84
- Pasteurella*,
 - vitamin requirements of, III, 155, 181
- Pellagra, IV, 93
 - beneficial effect of liver extract on, I, 283
 - coenzyme I and, IV, 98
 - death from, I, 60-62
 - etiology of, I, 163
 - manifestations of, I, 89-92; III, 10; IV, 97-100
 - in monkey, II, 71, 88, 89, 90
 - niacin and, I, 74, 89, 175, 182, III, 15, 33; IV, 95, 97, 98
 - porphyrin and, I, 175
 - in pregnancy, I, 78
 - riboflavin and, I, 88
 - ventriculin and, IV, 95
- Penicillic acid, antagonism of p-aminobenzoic acid to, II, 231
- Penicillin,
 - p-aminobenzoic acid and, II, 231
 - bacteriostatic activity of, II, 345
- Pepsin,
 - avitaminosis C and, I, 162
 - intrinsic factor and, I, 286
 - tissue irritation by, I, 38
- Peptic ulcer,
 - malnutrition and, I, 81
 - scurvy and, I, 95
- Peptone,
 - factor TS and, I, 263
 - growth media and, I, 251, 260
 - "Percorten," action on heart, V, 113
- Perhydrovitamin A,
 - synthesis of, II, 175-177
- Periodocasein, IV, 209
- Perosis, I, 40
 - choline and, I, 29, 40, 49, 182
 - manganese deficiency and, I, 40
 - methionine and, I, 182
- Peroxidase,
 - effect of goitrogenic agents on, V, 290
- Pfeiffer's bacillus*,
 - thiamine synthesis by, III, 28
- Phagocytosis,
 - vitamin deficiencies and, IV, 63, 64
- Phenol,
 - antisterility activity of, II, 120
- Phenolase,
 - relation of p-aminobenzoic acid to, II, 232, 233
- Phenylalanine,
 - dietary requirements, IV, 101
 - metabolism, I, 76, 184, 187
- p-Phenylenediamine,
 - effect on enzymes, II, 338, 339
 - on liver respiration, II, 338
- Phenylthiourea,
 - goitrogenic activity of, V, 276
- Phlorhizin diabetes, IV, 202
- Phosphatase,
 - bone regeneration and, V, 213
 - deposition of calcium and serum, IV, 110
 - effect of steroid hormones on, IV, 301
 - goitrogenic agents and, V, 292
 - tumors of prostate and, II, 355, 356
 - vitamin B₆ conjugate and, IV, 14
 - vitamin D and, IV, 110; V, 60

- Phosphatides,
 choline and, I, 13, 160, 165
 in liver fat, I, 46
 Phosphocreatine, I, 179
 Phospholipides,
 biological formation of, II, 11, 12
 choline and, I, 1, 7, 40, 45, 47, 49, 181
 lecithin and, I, 28
 metabolism of, I, 34, 47, 48, 50
 oxidation of, II, 324, 339
 Phosphorus,
 deficiency,
 effect on lactation, III, 77
 on litter, III, 77
 on reproduction, III, 76-78
 on rickets, III, 78
 on skeleton, III, 178
 metabolism of, I, 158, 175; III, 9, 15
 nutritional requirements, I, 70, 75, 76, 79
 radioactive, I, 49, 50
 vitamins and, III, 9, 18
 Phosphorylation, I, 171, 185, 186
 Photokinetic systems, of lower invertebrates, I, 214
 Photophobia,
 in malnutrition, I, 81
 riboflavin and, I, 88, 89
 Photoreception, I, 195
 animals and, I, 223, 224
 carotenoids and, I, 195, 196, 223, 225
 plants and, I, 223
 vitamin A and, I, 195
 Photosynthesis,
 carotenoids and, I, 198, 200-202
 chlorophyll and, I, 201, 203, 250
 evolution of oxygen in, I, 202, 211
 phyococyanin and, I, 203
 Phototaxis, I, 195, 203, 211, 213, 224
 Phthiocol, III, 214, 216
 synthesis by *Myobacteria*, III, 213-215
 Phycocyanin,
 absorption spectrum of, I, 202 ff.
 structure of, I, 206
 Phycoerythrin,
 absorption spectrum of, I, 204, 205, 206
 structure of, I, 206
Phycomyces blakesleeana, III, 111, 117, 124-126, 128, 129, 201
 action of thiamine on, III, 117, 124, 126
 growth factors for, III, 201
 Phytic acid,
 effect on vitamin D requirement, III, 18
 Phytochlorin, I, 198
 Phytol, I, 218
 carotenoids and, I, 199, 200
 distribution, I, 200
 lycopene and, II, 218
 Phytosterols, I, 336
 Pidgeon,
 vitamin requirement of, V, 169
 Pigmentation,
 p-aminobenzoic acid and, II, 216; II, 217
 Pigments,
 absorption spectra of, I, 197, 201, 204, 205, 207-209
 complexes with proteins, I, 206
 photosynthesis and, I, 201
 Pituitary,
 anterior,
 assay of extracts of, IV, 319-321
 diabetogenic action of, V, 213
 dysfunction of, II, 126
 effect of androgens on, II, 369, V, 326-329
 of castration on, V, 326
 of estrogens on, II, 356, 393-396
 of removal of, II, 357, 370, 392, 394, 396, 397
 of sulfaguanidine on, III, 63
 gonadotropic effect of extracts of, III, 235
 hormones of, see under name of individual hormones
 interaction with thyroid, IV, 194
 lactation and, IV, 141
 nutrition and hormone production, IV, 136
 synergism with gonadotropin, III, 357
 vitamin E deficiency and, III, 9
 diabetogenic activity of, V, 245
 effect of androgens on, V, 265-267, 357
 of castration on, V, 250
 of estrogens on, V, 243-246, 248-251, 261, 265-267
 goitrogenic agents and, V, 276, 279 ff., 315
 hormone of,
 estrus and, IV, 136
 production of, IV, 136
 testicular tumors and, II, 357, 369
 isolation of thyrotropin from, III, 64
 luteotropic factor of, V, 329
 psendohypophysectomy and, IV, 136
 vitamin A requirement and, IV, 144
 vitamins in, I, 241, 244
Pityrosporum ovale,
 growth factors for, III, 213

- Placenta,**
 activity of extracts of, I, 297, 302
 antipernicious anemia principle in, I, 283
 carotene and, IV, 81
 choline in, I, 13
 effect of extracts of, on birds, II, 392
 estrogens in, I, 326-329
 inadequate nutrition and, III, 74
 passage of immune bodies through IV, 40
 progesterone in, I, 296, 303
 vitamins and, II, 110, 114, 198; III, 81; IV, 81, 87, 101, 108
- Plant hormones,** see also Auxins, II, 342
- Plants,**
 containing goitrogenic agents, V, 276
 goitrogenic activity of, 275
 symbiosis and vitamin requirement of, III, 33
 vitamins in, II, 17, 33, 115
- Plasma,**
 albumin in human, IV, 75
 ascorbic acid in, I, 66, 98, 169, 184; IV, 103
 carotene in, II, 328; III, 82
 globulin in human, IV, 75
 pigments, I, 270
 vitamin A in, II, 328; III, 82; IV, 80
- Plasmodium cathemurium*,**
 biotin deficiency and resistance to, IV, 55
- Plasmodium lophurae*,**
 vitamins and resistance to, IV, 42, 43, 55
- Pneumococcus,**
 antiserum and, IV, 55
 resistance to,
 diet and, IV, 45, 46, 49
 vitamins and, IV, 45, 49, 54, 55
- Poliomyelitis,** susceptibility to,
 genetic, IV, 39
 nutrition and, IV, 43, 44
 vitamins and, IV, 43-45, 48
- Polycythemia,**
 cobalt, ascorbic acid and, I, 174
 liver extracts and, I, 281
- Polyneuritis,**
 amino acids and, I, 183
 circulatory manifestations in, I, 87
 enzymes and, I, 162
 liver extract and, I, 283
 nitrogen balance in, I, 169
 vitamin deficiency and, I, 91, 161, 163
- Polytomella caeca*,**
 reaction to thiamine, V, 94
- Polyuria,**
 vitamins and, I, 99
- Porphyryn caking,** III, 55
 sulfonamides and, III, 54, 57, 62
 vitamins and, III, 54, 57, 58, 62, 67
- Porphyropsin,**
 absorption spectrum of, I, 217, 218, 221
 carotenoid components in, I, 218
 in vertebrates, I, 222, 224
 vitamin A in, II, 202
 vitamin A₂ and, I, 167, 215, 216, 220, 224
- Potassium,**
 deficiency, effect on reproduction, III, 78
 ion, effect on choline acetylase, III, 366
- Potassium iodide,**
 goiter and, III, 79, 80
- Poultry,**
 vitamin requirements of, V, 164-171
- Precipitin,**
 vitamins and formation of, IV, 63
- $\Delta^4,8$ -Pregnadiene-17,21-diol-3,11,20-trione,**
 I, 336
- Pregnancy,**
 anemias of, I, 64, 282; V, 140 ff.
 calcium and, III, 77, 78; IV, 108
 corpus luteum and, I, 296
 diagnosis of, IV, 319
 effect of androgens on nausea and vomiting of, V, 373
 excretion of androgens during, I, 309, 310, 313, 314
 estrogens in, I, 327, 328, 332, 333, 335
 of progesterone in, I, 303
 of reduction products of progesterone in, I, 297-299, 313
 gonadotropin in blood during, III, 298, 301, 306, 307, 310, 323, 324, 327, 331
 iodine deficiency and, III, 79
 mineral requirements of, I, 64, 70; III, 78
 nutrition and, I, 75, 77, 81, 82; III, 74, 75, 82, 91, 92, 98; IV, 121
 protein and, III, 76, 93
 toxemia of, I, 80, 303; II, 129
 vitamin A requirement of, II, 203; III, 96
 vitamin deficiency and, III, 81, 82, 84, 90
- Pregnane,**
 chemistry of, I, 295, 296, 306, 350
 crystal structure of, II, 438, 451

- Pregnanediol**,
 configuration of, I, 296
 conversion of progesterone to, I, 299,
 300, 305, 315, 317, 322
 crystal structure of, II, 438
 effect on sodium retention, IV, 289
 excretion of, I, 301-304, 305
 isolation of, I, 297, 298, 300, 305
Pregnanediol glucuronide,
 excretion of, I, 298, 300-302
Pregnanedione, I, 298, 299, 317
Pregnanetriol,
 excretion, I, 314
Pregnanetriol A (Uranetriol),
 isolation of, I, 306
Pregnanetriol B, see 3(γ),16,20-Allopreg-
 nanetriol
3(α)-Pregnanol, I, 298, 299
**3(α)-Pregnanol-20-one (epipregnanol-
 one)**, I, 299, 317
 Δ^5 -Pregnene-3(β),20(α)-diol, I, 300, 317
 Δ^5 -Pregnene-3(β),21-diol-20-one, I, 326
 Δ^5 -Pregnene-17,20,21-triol-3-one, I, 326
 Δ^5 -Pregnenolone, IV, 277
 Δ^5 -Pregnenol-3(β)-one-20,
 from cholesterol, I, 336, 350
 relationship to progesterone, I, 319, 322
Premenstrual tension, IV, 152, 154, 160
 pathophysiology of, V, 356
 therapy with androgens, V, 357
Pressor substances, III, 230
Procarcinogen,
 p-aminobenzoic acid as, II, 344
 biotin as, II, 313, 333, 344
 inositol as, II, 344
 vitamin B complex as, II, 344
"Pro-estrogen," III, 319, 320
Progestational hormone,
 see Progesterone
Progesterone, III, 301
 androgenic effects of, I, 313
 avidin formation and, IV, 140
 biological conversion to 17-keloster-
 oids, I, 317
 of desoxycorticosterone to, I, 366, 367
 corpus luteum and, I, 296; V, 355
 crystal structure of, II, 436, 438
 effect on capon's comb, II, 395
 on nitrogen excretion, IV, 262, 277
 on sodium retention, IV, 289
 estrogen inactivation and, I, 333
 formation of, I, 335; III, 320
 isolation of, I, 297, 322, 303
 lactation and, IV, 141
 metabolism of, I, 295, 301, 302, 315,
 317, 322, 332, 337
 occurrence, I, 296
 reduction products of, I, 298-300, 305
 requirement for, I, 304
 "stable," I, 350
 vitamin E and, II, 327
Progesterin, I, 332
Prolactin, see also Lactogenic hormone,
 V, 218
 antagonism of estrone to, II, 392
Prolan, III, 298, 324
"Prolanoid," III, 316, 317
Promizole,
 effect on basal metabolic rate, V, 284
 on iodine retention, V, 288
 on pituitary, V, 280
 thyroid and, V, 279, 295
Prontosil, III, 230
Propionic acid bacteria,
 vitamins and, III, 118, 119, 135, 181
Propylene glycol,
 as solvent for androgens, V, 323
Propylthiouracil,
 therapeutic value of, V, 305
 toxicity of, V, 316
Prostate,
 estrogens and, II, 354-356; V, 251
 hypertrophy of, V, 425-427
 testosterone therapy in, V, 425
 phosphatase content of, II, 355
Prostigmine,
 effect on nerve, III, 372
Protein hormones, I, 178, 186; IV, 143
Protein(s), IV, 75, 76
 amino acids and, IV, 75
 biological fat synthesis from, I, 164;
 II, 1-8
 carcinogenesis and, I, 39
 choline and, I, 7; V, 78
 conjugated, I, 166
 contracted pelvis and, III, 75
 deficiency,
 agglutinin formation, IV, 64
 manifestations of, II, 331; IV, 76, 77
 phagocytosis and, IV, 65;
 undernutrition and, IV, 77
 derivatives of, I, 157, 288
 dietary, I, 60, 68, 77, 161, 163, 184
 ascorbic acid and, IV, 101
 phenylalanine requirement and, IV,
 101
 tyrosine requirement and, IV, 101
 digestibility of, I, 162
 foreign, I, 173, 174
 intestinal flora and, III, 23, 31, 44
 iodinated, IV, 207 ff.
 absorption spectra of, IV, 239

- bioassay of, IV, 227-232
 effect on body growth, IV, 244
 on egg production, IV, 246
 on feather growth, IV, 246
 on milk secretion, IV, 241
 of temperature on, IV, 217
 iodine content of, IV, 210
 preparation of, IV, 208, 214
 thyroidal activity of, IV, 211-222, 227
 thyroxine content of, IV, 222, 233
 lipotropic effect of, I, 18 ff., 35 ff., 44
 metabolism, I, 158, 165, 184; II, 8, 24; IV, 268
 in premature infant, IV, 77
 vitamin E and, V, 191
 pigments and, I, 198, 206
 plasma levels of, IV, 76
 precipitation of, I, 233
 reproduction and, III, 76, 77, 93, 94
 requirements, I, 68, 75, 76, 82
 in retinal cycle, I, 216
 sensitization by, I, 173
 serum, I, 83, 98
 corticotrophic hormone and, V, 225
 sources of, in infancy, IV, 76
 testicular deficiency and, V, 414
 tumors and, II, 317
 vitamins and, I, 157, 161, 163, 165, 166, 170, 184, 185, 231, 235; II, 9, 11; III, 7, 8, 17, 42; V, 181
 wound healing and, IV, 106
Proteus vulgaris,
 coenzyme and, III, 2, 192
 glutamine requirement of, III, 147
 inositol synthesis by, III, 30
 nicotinic acid and, III, 181-185
 vitamin synthesis by, III, 28-30
 Prothrombin, III, 55
 biotin and, II, 56
 folic acid and, III, 56
 sulfasuxidine and, III, 56
 vitamin K and, I, 103, 157, 176; II, 327; III, 52, 91; IV, 114-116
 Protochlorophyll,
 absorption spectra of, I, 205
 Protohemin,
 protozoan metabolism and, I, 262
 Protoporphyrin,
 as growth factor for protozoa, I, 262
 pantothenic acid and, I, 176
 synthesis, I, 279
 Protozoa, IV, 37
 growth factors for, I, 249-268
 resistance to,
 biotin deficiency and, IV, 55
 nutrition and, IV, 46
 Provitamin A, see also Carotene, I, 71
 Provitamin D₂, see Ergosterol
 Provitamin D₃, see 7-Dehydrocholesterol
 Provitamin D₄, see 22-Dihydroergosterol
 Provitamin(s),
 absorption spectrum of, I, 118
 Pruritus,
 use of androgens in senile, V, 381
 vulvae, use of androgens in, V, 381
Pseudomonas fluorescens,
 vitamin synthesis by, III, 28-30
Pseudomonas spp.,
 inositol metabolism and, III, 209
 "Pseudopyridoxine," I, 243
 Pterine, III, 262, 279
 Pteroylglutamates, V, 121, 122, 138
 hemopoietic activity of, V, 122
 Pteroylglutamic acid, see also Folic acid,
 V, 121-152
 action mechanism of, V, 151
 gastrointestinal absorption and, V, 131
 history of, V, 122 ff.
 liberation from pteroylheptaglutamate,
 V, 151
 modes of administration, V, 143 ff.
 relation to antipernicious anemia principle, V, 143
 response to, V, 47
 therapeutic value in diseases of infants
 and children, V, 152-154
 in pernicious anemia, V, 142-150
 in sprue, V, 125 ff., 129, 130
 thiamine synthesis and, V, 45
 Pteroylhexaglutamylglutamic acid, V,
 138
 Pteroyltriglutamic acid, V, 121, 122, 138
 Purines,
 absorption spectra of, I, 129
 antagonism to benzimidazole, III, 203
 to sulfonamides, III, 204-206
 as bacterial growth factors, III, 198 ff.
 effect on growth response to p-amino-
 benzoic acid, III, 201, 204-206
 Pyracin,
 requirement of chick, V, 168
 α -Pyracin,
 anemia and, IV, 18, 19
 growth and, IV, 18, 19
 vitamin B₁₀ and, IV, 16
 vitamin B₁₁ and, IV, 16
 β -Pyracin,
 anemia and, IV, 18, 19
 growth and, IV, 18, 19
 Pyridine nucleotides, III, 180 ff.
 bacterial synthesis of, III, 187, 189-191

- function in *Hemophilus* bacteria, III, 189
- Pyridine-3-sulfonic acid, III, 185, 191
- derivatives of, III, 197, 198
- effect on mice, V, 192
- as growth inhibitor for *Staphylococcus aureus*, III, 191
- Pyridoxal,
- growth activity in mice, V, 187
- vitamin B₁₀ and, IV, 16
- vitamin B₁₁ and, IV, 16
- Pyridoxamine,
- growth activity in mice, V, 187
- vitamin B₁₀ and, IV, 16
- vitamin B₁₁ and, IV, 16
- Pyridoxine (vitamin B₆), I, 34, 38, 87, 159; III, 63; IV, 54, 57
- absorption spectrum of, I, 116, 130, 138, 144
- assay of, I, 138
- bacterial synthesis of, III, 23, 29, 137-139, 143
- biological fat synthesis and, I, 164; II, 3, 4, 6, 9, 22
- breeding and, III, 87
- coenzyme function of, II, 17
- congenital malformations and, IV, 142
- deficiency, II, 5, 21, 23; III, 7, 8
- manifestations of, I, 85, 92, 93, 165; V, 185
- oviduct growth and, IV, 137
- phagocytosis and, IV, 65
- resistance to lobar pneumonia and, IV, 55
- distribution of, I, 234 ff., 243, 244; II, 335, 336
- α -estradiol and, IV, 138
- effect on protein appetite, I, 161
- as growth factor for bacteria, III, 137 ff., 191; IV, 2, 3, 18
- for fungi, III, 142-145
- for protozoa, I, 256, 258
- in hemoglobin regeneration, I, 175
- identification of, I, 144
- interrelations with other vitamins, III, 7, 8, 32, 33
- lactation and, IV, 141
- liver fat and, I, 31, 32, 35
- metabolism of, II, 21
- refection of, III, 52
- requirements,
- of mice, V, 185-188
- of poultry, V, 167, 170, 171
- thyroid and, IV, 139
- structural specificity of, III, 145
- tumors and, II, 230, 320
- vitamin M deficiency and, II, 91
- Pyrimidine(s),
- absorption spectrum of, I, 126 ff.
- fluorescence of, I, 145
- as growth factor for fungi, III, 202
- for *Neurospora crassa* mutants, III, 202
- for protozoa, I, 252 ff., 265
- Pyriethamine, III, 129-132
- antagonism to thiamine, III, 129-132
- resistance of *Escherichia coli* to, III, 130
- Pyrocalciferol,
- crystal structure of, II, 435, 454, 455
- Pyruvates,
- in blood in thiamine deficiency, II, 17
- effect of vitamins on utilization of, II, 3
- oxidation in liver, II, 35
- Pyruvic acid, II, 15, 275
- beriberi and, IV, 88
- in blood, I, 9, 100
- carboxylase and, IV, 88; V, 115
- decarboxylation of, I, 171
- thiamine and, II, 23, 317; III, 6; IV, 86

Q

- Quinolinic acid, III, 183, 185
- pyrazine analogs of, III, 186
- Quinones,
- antisterility action of, II, 120
- Quinoxaline, II, 52, 55, 57

R

- Radioiodine, IV, 238
- Rape seed,
- effect on thyroid, V, 276, 281, 306
- on thyrotropin content of pituitary, V, 294
- goitrogenic activity of, V, 276 ff.
- Rat,
- dental caries in, II, 256-265, 293-297
- effect of corticotropic hormone on hypophysectomized, V, 215
- of growth hormone on hypophysectomized, V, 200
- of inanition on pregnant, III, 74
- of purified diets on, III, 24
- egg white injury in, II, 30, 31, 63
- "plateaued," V, 200
- relative growth rates of, V, 175, 176
- vitamin requirements of, II, 34, 143; III, 29
- vitamin synthesis in, III, 28, 29
- Redox catalyst,

- riboflavin as, III, 5
- Reductinic acid, I, 260
- Reductone, see Glucic acid
- Refection, III, 23, 25-29, 51; V, 66
 - diet and, III, 26, 51
 - intestinal synthesis and, III, 26, 31, 50, 51
 - of vitamins, III, 52
- Reproduction,
 - p-aminobenzoic acid and, IV, 139
 - avidin-biotin complex and, IV, 140
 - calcium and, III, 76-78
 - carbohydrates and, III, 76
 - choline and, III, 88
 - diet and, III, 74, 98
 - effect of androgens on, II, 361
 - of estrogens on, II, 390
 - of vitamin E on, II, 108
 - inositol and, IV, 139
 - phosphorus and, III, 77, 78
 - proteins and, III, 76, 77
 - vitamin deficiency and, III, 81, 85, 88-91
 - vitamins B and, IV, 135
 - yeast and, IV, 136
- Reproductive organs,
 - effect of androgens on, II, 361
 - of estrogens on, II, 361, 390
- Reticulocytes,
 - liver extracts and, V, 145
 - pernicious anemia and, I, 270, 272-278, 281, 284, 287
 - response to pteroylglutamic acid, V, 145
 - to thiamine, V, 145
 - xanthopterin and, IV, 26
- Reticulocytosis, I, 282
- Retina, I, 215
 - carotenoids proteins in, I, 166, 197, 223, 231
 - globules of, I, 223
 - photoreceptor systems of, I, 218
 - rods of neurokeratins in, I, 167
 - spectra of, I, 217, 218
 - vitamins A and, I, 186, 195, 214, 218, 219, 221, 222, 225, 240
- Retinene (visual yellow), I, 167, 217
 - identity with vitamin A aldehyde, III, 6
- Retinene₁, I, 215-217, 219, 224
- Retinene₂, I, 216, 218
- Retinine, see Retinene
- Rhizobia,
 - biotin or coenzyme R requirements of, III, 159, 163-165
 - vitamin synthesis by, III, 135, 163
- Rhodopin, I, 196
- Rhodopsin (visual purple), II, 202
 - absorption spectrum of, I, 219
 - distribution of, I, 167, 223
 - properties of, I, 167
 - system, I, 215-218, 220-223
- Rhodopurpurin, I, 196
- Rhodovibrin, I, 196
- Rhodoviolascin, I, 196
- Riboflavin (vitamin B₂), I, 159; III, 63, 132 ff., IV, 54, 56, 91-95
 - absorption spectrum of, I, 116, 129; V, 69
 - adrenaline and, I, 178
 - alanine and, I, 183
 - amino acid oxidase and, I, 166, 171
 - as anticarcinogen, II, 344
 - assay of, I, 138, 144, 146, 148-151, 233; III, 135
 - bacterial synthesis of, III, 23, 31, 32, 39-42, 96, 135, 136
 - biological fat synthesis and, II, 4, 9
 - biotin and, II, 13
 - breeding and, III, 87, 88
 - carbohydrate metabolism and, III, 6, 17; IV, 91
 - carotenoid pigments and, V, 42
 - coenzyme function of, II, 17
 - conversion to lumichrome, III, 3
 - coprophagy and, III, 96
 - dark adaptation and, III, 5, 6, 17
 - deamination of amino acids and, III, 7
 - deficiency, I, 63, 65, 83, 162, 164, 166, 172, 182
 - anemia and, IV, 142
 - effect on embryo, III, 96, 97
 - estrus and, III, 84, 96; IV, 139, 144
 - manifestations of, I, 66, 86-89, 101, 182; III, 85-88, 96, 97; IV, 92, 93, 143
 - phagocytosis and, IV, 64
 - resistance to bacterial infection and, IV, 42, 43, 45, 54, 55
 - in diet, I, 73, 74, 75, 76, 82, 163; IV, 142; V, 68
 - distribution, I, 79, 234, 238, 239, 244; II, 315, 316, 336; III, 6, 7; IV, 91-93, 142, 143
 - effect on food utilization in mice, V, 181
 - on luminescent bacteria, III, 135
 - of phosphate on activity of, I, 251
 - on protein appetite, I, 161
 - enzyme systems and, IV, 91
 - estrogens and, IV, 138, 149
 - excretion of, III, 15, 39; IV, 92; V, 68

- as growth factor for protozoa, I, 256-258, 262
 in hemoglobin regeneration, I, 175
 interrelations with other vitamins, I, 235; III, 4-8, 17, 32
 lactation and, IV, 141
 phenazine analog of, V, 192
 protein intake and, I, 165; III, 8; V, 68.
 requirements,
 of bacteria, III, 133-136
 of chicks, V, 166, 170, 171
 in infancy, IV, 92
 of mice, V, 182
 structural specificity of, III, 136
 tumors and, I, 39; II, 308, 313, 315, 316, 318, 328
 visual purple and, III, 4, 6, 17
 vitamin M deficiency and, II, 91
 Riboflavin phosphate, I, 166
 Riboflavin tetraacetate, III, 136
 Rickets, I, 95, 96, 103, 171
 blood serum in, IV, 109, 110
 deaths from, I, 61
 dental caries and, II, 284
 effect of calcium-phosphorus ratio on, III, 78, 79
 of phosphorus deficiency on, III, 78
 in monkeys, II, 71-79
 manifestations of, I, 96, 103; IV, 111, 112
 prevalence of, I, 59, 60, 62, 66
 susceptibility of premature babies to, IV, 111
 therapeutic effect of cod liver oil, II, 156
 of irradiation, II, 76, 284
 vitamins and, I, 95; II, 156; III, 89; IV, 107, 108
 Roots,
 effect of thiazoles on, III, 126
 inhibitory effect of sulfonamides on, III, 177
 vitamin requirements of, III, 121, 122, 124-128, 142, 143, 182, 183
 Rumen,
 synthesis of vitamins in, I, 159
 Ryzamin-B, I, 24, 180
- S**
- Saliva, dental caries and, II, 279-281, 290
 Salmonella,
 effect of nicotinic acid on, III, 181, 184
Salmonella enteritidis, IV, 59, 60
 diet and resistance to, IV, 45, 49
 susceptibility of mice to, IV, 41
Salmonella paratyphi,
 protein deficiency and agglutinin production with, IV, 63
 Salts,
 effect on vitamin stability, V, 84
 Sapogenin, I, 323
 Sarcinene, I, 223
 Sarcoma,
 in chick due to ferric chloride intake, II, 138
 vitamins and, II, 309, 311, 316, 318-320, 325
 Sarcoma virus,
 nutrition and susceptibility to, IV, 42
 Sarcosine, I, 26, 29
 Sarmentogenin, I, 371
 Scurvy, I, 97; IV, 63, 103, 104
 ascorbic acid and, I, 94, 95, 102; II, 7, 81, 83, 84
 blood serum in, IV, 103
 cod liver oil and, III, 16
 congenital, III, 89
 deaths from, I, 60, 61
 immunological aspects of, IV, 63
 manifestations of, I, 94, 95, 174; II, 79, 80; IV, 103, 104, 106
 in monkeys, II, 79, 80, 86, 102
 nitrogen metabolism and, I, 164
 pregnancy and, III, 88
 vitamin A and, III, 16
 Sea urchin,
 action of thiourea on, V, 283
 Seminal duct,
 vitamin content of, I, 237
 Seminal vesicles,
 effect of corticotropic hormone on, V, 224
 of estrogens on, V, 251-259
 Seminiferous tubule,
 failure of, V, 410
 Serine, I, 19, 29, 30
 and cephaline, I, 47, 166
 decarboxylation of, I, 160
 effect on liver lipides, I, 47
 Serum, blood,
 albumin, determination of, I, 98
 ascorbic acid and complement of, IV, 64
 calcium in, II, 78
 immune bodies in, IV, 40
 iodinated proteins of, IV, 213, 214, 222, 225
 phosphatase,
 effect of ascorbic acid on, I, 171
 of vitamin D on, II, 324

- phosphorus level of, II, 76
- proteins,
 - determination of, I, 98
 - in rickets, I, 103
- Sex behavior,
 - effect of androgens on, in birds, II, 369, 370
- Sex differentiation,
 - effect of estrogens on, V, 241, 242
- Sex hormones, see also Androgens, Estrogens and under name of individual compounds
 - as carcinogens, II, 353, 354
 - crystal structure of, II, 436-441
 - intermediate metabolism of, I, 293-343
 - structure of, II, 252, 436, 441
- Simmonds' disease, IV, 266, 271, 286, 294
 - effect of androgens on, V, 376
- α -Sitostanol,
 - crystal structure of, II, 454, 455
- Sitosterol, I, 261, 324
- β -Sitosterol,
 - crystal structure of, II, 456
- γ -Sitosterol,
 - crystal structure of, II, 435, 453, 454, 455, 456
- β -Sitosterol acetate,
 - crystal structure of, II, 453-455
- Skeleton,
 - effect of estrogens on, V, 236
 - of mineral intake on, III, 78, 79
 - of parathyroid on, III, 78
 - infantile scurvy and, III, 9
 - vitamin deficiency and, III, 85-89, 96, 97
- Skin,
 - effects of estrogens on, V, 238, 381
 - of fatty acid deficiency on, III, 11
 - pellagra and, I, 175, 182; IV, 97, 99
 - scurvy and, IV, 105
 - vitamin deficiency and, I, 83, 87, 94; II, 30, 202; III, 10, 11
 - vitamins in, I, 237
- Sodium,
 - deficiency, reproduction and, III, 78
 - excretion, effect of steroid hormones on, IV, 292, 346, 353
 - loss of, in dehydration, IV, 79
- Sodium azide,
 - effect on thyroid hormone formation, V, 289
- Sodium homo-cysteinate, I, 26
- Sodium sulfate,
 - effect on resorption of gonadotropin, III, 323
- Sodium sulfide,
 - effect on thyroxine formation, V, 289
- Spermatogenesis, II, 369, 393, 396, 397; V, 404
 - effect of exogenous testosterone on, V, 415
- Sphingomyelin, I, 47, 165
- α -Spinastanol,
 - crystal structure of, II, 454, 455
- β -Spinastanol,
 - crystal structure of, II, 435, 454, 455
- α -Spinasterol acetate
 - crystal structure of, II, 435
- γ -Spinasterol acetate,
 - crystal structure of, II, 453
- Spirilloxanthin, I, 196
- Spleen,
 - aerobic respiration of, I, 239
 - anaerobic glycolysis in, I, 239
 - effect of prenatal starvation on, III, 74
 - estrogen in, IV, 137
 - extrinsic factor in, I, 286
 - gonadotropin inactivation and removal of, III, 309, 310
 - implantation of steroid pellets in, IV, 149, 151
 - phagocytic cells in, II, 136
 - vitamins in, I, 236, 237, 240; II, 117, 319
- Sprue, I, 80, 284; V, 124-139
 - antipernicious anemia factors in livers of patients with, I, 283
 - symptoms of, V, 124 ff.
 - therapeutic effect of liver extracts, I, 282; V, 130
 - of pteroylglutamates in, V, 125, 130 ff., 154
 - of thiamine in, V, 154
 - vitamin M deficiency and, V, 125
- Staphylococcus albus*,
 - vitamin B synthesis by, III, 139
- Staphylococcus aureus*,
 - inhibition by antibiotic factor, III, 169
 - by pyridine-3-sulfonic acid, III, 191
 - by sulfonamides, III, 177, 196
 - uracil requirement of, III, 198
 - synthesis by, III, 140, 198
- vitamins and, III, 118, 119, 121, 124-126, 136, 165, 181
- Staphylococcus flavus*,
 - growth factors for, III, 118
 - synthesis of riboflavin by, III, 136
- Starch,
 - effect on intestinal vitamin synthesis, III, 31
 - refection and, III, 26
- Starvation,

- compared to thiamine deficiency, V, 114
- Sterility, II, 124-129
- diet and, IV, 136
- effect of phosphorus deficiency, III, 77
- vitamins and, II, 142, 143; III, 85, 90, 91, 96; IV, 136, 168
- Steroid hormones, see also under Sex hormones, Androgens, Estrogens, and under name of individual hormones
- absorption spectra of, I, 137
- assay of, I, 110
- biosynthesis of, I, 294
- carcinogenesis and, I, 295
- effect on sodium excretion, IV, 292, 346, 353
- metabolism of, I, 294-338
- oxidation of, I, 324
- pellet implantation of, IV, 149, 151
- presence in pregnancy urine, I, 297
- protein anabolism and, IV, 256-303
- renotropic effect of, IV, 299
- Sterols, I, 110, 337; II, 12, 13, 447-450
- cholestenone and, I, 323
- configuration of, II, 450
- crystal structure of, II, 409-458
- distribution of, II, 458
- as growth factor for protozoa, I, 261
- sex hormones and, II, 440
- vitamin D and, I, 231
- Stilbene,
- estrogenic activity of, III, 232
- Stilbestrol, see also Diethylstilbestrol
- avidin formation and, IV, 139, 140
- diet and, IV, 137, 138
- effect on carcinoma of the breast, II, 357
- on prostate, II, 356
- estrogen response and, IV, 137
- folic acid and, IV, 144
- metabolic effects of, IV, 277
- structure of, IV, 258
- Stillbirths,
- iodine deficiency and, III, 79
- nutrition and, III, 74, 75, 92, 95; IV, 74
- vitamin deficiency and, III, 84, 88
- Streptogenin,
- effect on mice, V, 191
- Streptococcus fecalis*,
- glutamine requirement of, III, 148
- riboflavin requirement of, III, 133
- Streptococcus lactis*,
- stimulation by uracil, III, 199
- synthesis of folic acid by, III, 60, 61
- thymine as nutrient for, III, 200
- vitamin B requirement of, III, 134, 139
- Streptococcus lactis* R,
- vitamins as growth factors for, IV, 4, 8, 10, 11, 15
- Streptococcus lactis* R factor, IV, 4, 7, 8, 19-22
- folic acid and, IV, 8, 19-21
- hematopoiesis and, IV, 12
- L. casei* and, IV, 7
- leucopenia and, IV, 8
- succinylsulfathiazole and, IV, 23
- vitamin B₂ conjugate and, IV, 12
- vitamin M and, IV, 19-21
- xanthopterin and, IV, 27
- Streptococcus salivarius*,
- thiamine and, III, 118, 129
- Streptococcus spp.*,
- and pyridoxine, III, 138
- Streptococcus viridans*,
- glutamine requirement of, III, 146, 148
- Streptococcus zymogenes*,
- glutamic acid requirement of, III, 146
- Streptococci, hemolytic,
- action of sulfonamides on, III, 172, 175
- adenine and, III, 200
- adenylic acid and, III, 141, 200
- growth factors for, III, 134, 140, 141
- glutamine requirement, III, 146, 150
- inhibitory action of iodine on, III, 215
- pantoic lactone requirement, III, 155
- pantothenic acid requirement, III, 152, 154
- riboflavin requirement, III, 133
- stimulating action of uracil, III, 199
- vitamin M deficiency and resistance to, IV, 57
- Strophanthidin,
- crystal structure of, II, 441, 442, 445, 446
- Strychnine,
- affinity to choline esterase, III, 372
- Sub-vitamin A, I, 117, 133
- Succinic dehydrogenase, I, 166, 172
- Succinoxidase, I, 166
- effect of goitrogenic agents on, V, 291
- Succinylsulfathiazole, see Sulfasuxidine
- Sucrose, intestinal vitamin,
- synthesis and, III, 31, 32, 39
- Sugar,
- dental caries and, II, 264-267
- Sulfadiazine, III, 64, 67
- antagonism, to p-aminobenzoic acid, III, 56, 68
- basal metabolism and, III, 69
- diet and activity of, III, 49; V, 300
- effect on thyrotropin, V, 294

- L. casei* factor and, III, 56
 thyroid and, III, 63; V, 276, 281, 282
 toxic effects of, III, 53, 54, 56, 57, 65;
 IV, 23
 vitamins and, III, 56, 57
- Sulfaguanidine,
 p-aminobenzoic acid, III, 34, 53, 54,
 56; IV, 22, 23
 bacteriostatic action of, III, 53; IV,
 22
 effect on acetonitrile activity, V, 293
 on basal metabolic rate, III, 63; V,
 284
 on body growth, IV, 22; V, 283
 on food intake, V, 283
 on proteolytic enzymes, V, 277
 goitrogenic activity of, V, 276, 315
 liver extract and, III, 34, 53, 54
 norit eluate factor and, IV, 24
 nutrition and, III, 49, 53
 response to, V, 287
 thyroid and, III, 53, 63; IV, 22; V,
 277, 279, 292
 toxic effects of, III, 54, 56, 57, 62; IV,
 22
 vitamins and, III, 29, 30, 31, 34, 41,
 54, 56, 57; IV, 23
- Sulfanilamide, III, 229, 230
 antagonism to purines, III, 204-206
 goitrogenic activity of, V, 276
 nutrition and, III, 49
 reversal of inhibition by, III, 171-173,
 175
 thyroid and, III, 6
 toxic effects of, III, 56, 57, 64; IV, 28
- Sulfanilic acid,
 goitrogenic activity, V, 277
- Sulfanilylguanidine, see Sulfaguanidine
- Sulfapyrazine,
 hypoprothrombinemia due to, III,
 54, 56
 nutrition and, III, 49
 vitamin K deficiency due to, III, 56
- Sulfapyridine, III, 64, 195, 196
 antagonism to coenzyme, I, III, 196
 effect on thyroid, III, 63
 goitrogenic activity of, V, 285
 nutrition and, III, 49
 toxic effects of, III, 54, 57
 vitamins and, III, 54, 57
- Sulfathiazole,
 effect on heart function, V, 285
 nutrition and, III, 55, 63
 synthesis of folic acid and, IV, 23
 thyroid and, III, 63
 toxic effects of, III, 55-57; IV, 23
- vitamin K deficiency and, III, 56
- Sulfasuxidine,
 p-aminobenzoic acid and, III, 34, 68,
 69; IV, 23, 24
 nutrition and, III, 49
 synthesis of *S. lactis* R factor and, III,
 23
 toxic effects of, III, 54, 57, 58, 62, 64;
 IV, 22, 23
 vitamins and, III, 34, 41, 54, 56-58,
 62; IV, 23, 24
 xanthopterin and, IV, 26
- Sulfonamides, III, 64; V, 286 ff.
 absorption of, III, 65
 p-aminobenzoic acid and, II, 215, 216,
 220, 231; III, 56
 antagonism to methionine, III, 178
 bacteriostatic action, III, 24, 43, 44,
 68, 69, 170-180, 196
 diet and, III, 49; V, 300
 effect on bacterial vitamin,
 synthesis, II, 345; III, 23, 30, 32,
 33-35, 41, 50, 66, 68
 on basal metabolism, III, 69
 on enzymes, V, 290, 291
 on thyroid hormone, III, 64; V, 289
 excretion of, III, 65
 hematopoietic factors and, IV, 2, 22-25
 iodine and, V, 293
 liver and, III, 34, 54, 68
 melanine formation and, V, 292
 mode of action of, III, 55
 resistance to, III, 179
 toxic effects of, II, 132, 222; III, 54,
 58, 64-66, 68, 69; V, 74, 286, 295,
 297, 298
 vitamins and, III, 49-72
- Suprasterol I, 134
- Suprasterol I,
 crystal structure of, II, 454, 455
- Suprasterol II,
 crystal structure of, II, 435, 454, 455
- Symbiosis,
 effect on insect nutrition, III, 184
 on vitamin requirements of micro-
 organisms and plants, III, 33
- Synapses, III, 339 ff.
 choline esterase concentration in, III,
 344-350
 transmission of stimulus at, III, 360,
 373

- Tadpoles**,
 effect of vitamin E on pituitary gland,
 of, II, 116
- Tannic acid**,
 effect on resorption of gonadotropin,
 III, 323
- Tar**, carcinomas induced by, II, 311,
 326
- Taurine**, I, 162
- Taurocholic acid**, I, 162
- Teeth**,
 prenatal nutrition and, III, 95
 vitamins and, III, 9, 10, 13, 81
- Testalolon**, I, 307, 308
- Testes**, I, 239, 308
 atrophy, V, 410
 in cirrhosis of liver, IV, 148, 154,
 163, 165
 in malnutrition, IV, 148, 154
 biopsy, V, 398
 degeneration, due to arginine defi-
 ciency, II, 146
 vitamin E deficiency and, II, 122, 123,
 125
 effect of androgens on, V, 417, 418
 of estrogens on, II, 389, 390, 393
 on beak color, II, 371
 on plumage, II, 368, 373
 estrogens in, I, 326, 327, 329; V, 235
 extract of, I, 320, 321
 metabolic effects of, IV, 257
 hormone, see also Testosterone, I, 308,
 316, 321
 vitamin B deficiency and, IV, 136
 lipids in, I, 307
 prenatal starvation and, III, 74
 tumors of,
 diagnosis by urinalysis, II, 357
 gonadotropic substances and, II, 353
 hormones in, II, 357
 vitamins in, I, 232, 236, 237
- Testosterone**, II, 364
 activity of, II, 378, 380-382
 atrophy of sex organs due to, II, 354
 biological conversion of Δ^4 -androstene-
 dione to, I, 325
 to 17-ketosteroids, I, 326
 crystal structures of, II, 436, 438
 effect on adrenalectomized animals, IV,
 261
 on birds, II, 356, 366, 368, 369, 372-
 374, 376, 378, 383, 385, 403
 on electrolyte retention, IV, 289
 on estrogens, V, 264, 267
 on nitrogen retention, IV, 261, 270
 on seminiferous tubules, V, 399
 on spermatogenesis, V, 415
 esters of, II, 365, 382-384
 formation of, I, 314, 321, 335
 hypogonadism and, V, 411 ff.
 metabolism of, I, 315, 317, 318
 modes of administration, V, 411 ff.
 relative activity of, V, 319-325
 structure of, I, 307, 321
 synthesis of, I, 319
- Testosterone acetate**,
 activity of, II, 382-384
- Testosterone propionate**, II, 383
 clinical use of, IV, 264-270; V, 339-381,
 411 ff.
 creatinuria and, IV, 281
 effect on birds, II, 368, 369, 371, 372,
 382-385
 on electrolyte excretion, IV, 290, 291
 on endometrium, V, 329, 330
 on metabolism, IV, 294
 on sodium retention, IV, 289
 on tissue formation, IV, 298
 on urinary nitrogen excretion, IV,
 263-270
 modes of administration, II, 370, 378,
 384; V, 411 ff.
 relative activity, V, 319-325
- Testriol**, I, 307, 308
- Tetany**,
 blood calcium and, IV, 110, 113
 blood phosphorus and, IV, 113
 in infants, IV, 110, 113
- Tetrahydroxyquinone**,
 bacterial synthesis of, III, 209
- Thallium salts**,
 inhibitory effect on estrus, III, 329
- Theelin**, see Estrone
- Theelol**, see Estriol
- Thiaminase**, V, 56
- Thiamine (vitamin B₁, Aneurine)**, I,
 59, 87, 92, 127, III, 63; V, 105 ff.
 absorption of, III, 38
 absorption spectra of, I, 116, 126-129;
 V, 99
- acetylcholine** and, III, 367; V, 96, 109,
 113
- adrenaline** and, I, 178
- analogues** of, II, 342; III, 124-131
 effect on mice, V, 192
- antagonism** to pyriethamine, III, 129-
 132
- assay** of, I, 138, 145, 147-151
- biosynthesis** of, I, 159, 257; III, 23, 26-
 28, 31, 35-39, 42, 50, 51; IV, 86
- carbohydrate deficiency** and, II, 17, 18
 metabolism and, I, 158; IV, 86

- carboxylase and, I, 171; II, 317; IV, 86
casein and, II, 9
choline and, II, 12
congenital malformations and, IV, 192
deficiency, I, 73; II, 17, 20, 87, 88, 330;
III, 7, 8, 33, 84, 132
manifestations of, I, 83-87, 94, 101,
161, 162, 164, 168, 183, 184; III,
10, 25, 26, 50, 84; IV, 88-91;
V, 176-178
phagocytosis and, IV, 65
resistance to bacterial infection and,
IV, 43, 44, 49, 55, 62
derivatives of, III, 121-128
in diet, II, 3, 5, 23, 318
distribution of, I, 79, 234, 244, 283; II,
318, 329, 335, 336; IV, 86, 87, 89;
V, 103, 105-110
effect of acid on, II, 5
on fat synthesis, II, 3-9, 11, 16
on flagellates, III, 123
on *Phycomyces*, III, 117, 124, 126
on protein intake, I, 161
on reproduction, III, 84, 92
estrogens and, IV, 138, 139, 149
estrus and, IV, 139, 144
excretion of, I, 165; IV, 86, 89; V, 52
in feces, III, 27, 35-38
as growth factor for protozoa, I, 253,
255, 256, 258, 262, 264, 265
hyperthyroidism and, V, 64
interrelations with other vitamins, II,
91, 342; III, 6, 7, 15, 32, 33, 39
lactation and, IV, 140, 141
liberation by cholinergic nerve, V, 114
in nervous excitation, V, 96-105,
111
metabolism of, II, 318
photochemical sensitivity of, V, 102
physiology of, IV, 86, 87
in poisoned nerve, V, 115
pyruvic acid and, III, 6; IV, 86
refection and, III, 31, 52
requirements, I, 71, 75, 76, 177, 253;
III, 12; IV, 87, 88
of bacteria, III, 118, 121, 122, 129,
134, 165, 166, 209
of birds, V, 166, 169-171
of fungi, III, 33, 117 ff., 122, 123
of mice, III, V, 176 ff.
of roots, III, 28
thyroid and, IV, 139
of yeasts, III, 117, 123, 124
specificity of, III, 124-127
transformation to thiochrome, V, 104,
105
treatment of hypervitaminosis with,
III, 16
tumors and, II, 317, 318, 320
Thiamine-antithiamine complex, I, 168
Thiaminoprotein enzymes, I, 138
Thiazole-5-carboxylic acid, III, 197
Thiazole derivatives,
activity of, III, 126-128
Thiochrome, I, 100, 145, 147, 149, 150,
252
conversion of thiamine into, V, 105
Thiocyanates,
effect on iodine metabolism, V, 288
goitrogenic activity of, V, 275
inhibitory effect on thyroxine forma-
tion, V, 288
Thioglycolic acid, I, 184, 260
Thiohistidine betaine, I, 29
Thioneine, I, 183, 184
Thiouracil, IV, 230, 231, 245
adenoma and, V, 306
administration prior to thyroid re-
moval, V, 306
adrenotropic hormone and, V, 295
autonomic nervous system and, V, 299
bone deformities caused by, V, 286
effect on amphibian metamorphosis, V,
282
on basal metabolic rate, V, 284
on blood cells, V, 297
on body growth, V, 284-286
on bone marrow, V, 298
on enzymes, IV, 239; V, 290, 291
on fat deposition, V, 285
on kidney, V, 297
on liver, V, 297
on pituitary, V, 280
on plasma protein, V, 285
on reproductive system, V, 296
on serum albumin, V, 287
glutathione compared with, V, 293
goitrogenic activity, V, 277, 315
interaction with acetoneitrile, V, 293
iodine and, V, 288, 300
metabolism of, V, 301
periarteritis nodosa due to, V, 299
sex differences in response to, V, 299
therapeutic value in Grave's disease,
V, 305
thyroid and, IV, 202, 239; V, 279, 289
thyrotropin and, V, 294, 299
Thiouracil compounds,
therapeutic value in hyperthyroidism,
V, 305
Thiourea, III, 64
adenocarcinoma caused by, V, 279

- derivatives, adrenaline and, V, 296
 effect of dietary factors on activity of, V, 300
 on melanine formation, V, 292
 reduction of iodine by, V, 293
 relative activities of, V, 303
 thyroid neoplasmas caused by, V, 306, 315
 effect on adrenal cortex, V, 295
 on amphibian metabolism, V, 281
 on basal metabolism, V, 284
 on body growth, V, 284, 286
 on enzymes, V, 290-292
 on fat deposition, V, 285
 on heart function, V, 285
 on iodine metabolism, V, 287
 on liver, V, 286, 297
 on moulting, V, 282
 on pituitary, V, 280
 on plasma protein, V, 285
 on reproductive organs, V, 296
 on secondary sex characteristics, V, 286
 on serum albumin, V, 287
 goitrogenic activity, V, 277 ff., 315
 interaction with acetonitrile, V, 293
 iodine and, V, 288, 300, 315
 mechanism of antithyroid action, V, 293
 metabolism, V, 301
 milk production and, V, 296
 therapeutic value in Grave's disease, V, 305
 thyroid and, III, 63; V, 277, 280, 281, 284, 289, 293, 295, 306, 315
 toxicity of, V, 283, 297, 299
 Threonine, I, 29
 Thrombin, I, 176
 Thromboplastin, I, 103, 165, 176
 Thymine, V, 154, 155
 folic acid and, V, 45, 154
 as growth factor for *S. lactis* R, IV, 8
 therapeutic value in pernicious anemia, V, 123, 154
 in sprue, V, 154
 Thymus, I, 33, 170, 181
 effect of corticotropic hormone on, V, 225
 prenatal starvation and, III, 79
 pseudohypophysectomy and, IV, 136
 Thyroglobulin,
 analysis of, IV, 238
 Thyroid, I, 177, 240; II, 128
 adenomas of, V, 278, 279
 congenital goiter and degeneration of, III, 80
 diabetogenic action of, IV, 192-195
 dysfunction, testosterone and, IV, 272
 vitamin E deficiency and, II, 127, 146
 effect of, deficiency on feathers, II, 373
 on egg production, IV, 246
 on islet of pancreas, IV, 195
 on milk secretion, IV, 241
 of rape seed feeding on, V, 276
 resorption of gonadotropin, III, 323
 on sugar absorption, IV, 188, 196
 of thiouracil on, V, 315
 on vitamin requirements, IV, 139
 factors influencing the, V, 274
 hormone, see also Thyroxine, II, 127, 228
 basal metabolic rate and, III, 64
 effect of thiourea on secretion of, V, 292
 formation of, V, 285
 as regulator of thyrotropin, V, 293
 sulfonamides and, IV, 169-171
 temperature and requirement for, V, 300
 hyperemia, III, 55, 64, 67
 sulfonamides and, III, 63
 hyperplasia, II, 127; III, 64, 79
 goitrogenic agents and, V, 280, 281
 thyrotropin and, V, 294
 hypertrophy, III, 55, 63, 67, 79
 interaction with anterior pituitary, IV, 194
 iodine and, III, 79, 98; IV, 121, 207; V, 288, 289
 pseudohypophysectomy and, IV, 136
 proteolytic enzymes of, V, 292
 substance,
 effect on goitrogenic activity, V, 279
 sulfonamides and, III, 53, 63; IV, 22
 therapy,
 basal metabolic rate and, IV, 169-171
 thiamine and, IV, 169-171
 thiourea and, III, 63; V, 277, 280, 281, 284, 286, 289, 293, 295, 315
 vitamin A deficiency and, II, 199
 Thyroidectomy,
 effect on activity of growth hormone, V, 208
 on diabetes, IV, 199-201, 203
 on respiratory quotient, IV, 191
 thiouracil feeding compared with, V, 287
 Thyrotropic hormone, I, 177
 action on phlorhizinized rat, IV, 202
 assay of, IV, 331, 332

- effect on glycogen storage, IV, 190
on insulin secretion, IV, 195
Thyrotropin, see also Thyrotropic hormone, III, 64
action of goitrogenic agents on, V, 294, 295
activation by thiouracil, V, 295
of thyroid by, V, 293
blood levels of, V, 294
effect on amphibian metamorphosis, V, 282
on basal metabolic rate, V, 284
on iodine uptake, V, 288
on thiouracil content of thyroid, V, 302
factors regulating formation and release of, V, 293, 294
proteolytic enzymes and, V, 292
thiouracil compared with, V, 294
thyroid and, V, 294
Thyroxine, see also Thyroid, hormone, I, 157, 176, 177; III, 64
antagonists of, V, 316
bioassay of, IV, 227-232
effect on basal metabolism, V, 285, 293
on blood sugar level, IV, 188
on glycogen storage, IV, 190
on insulin secretion, IV, 195
on iodine accumulation, V, 288
on milk secretion, IV, 241
on thyroid, V, 281
formation,
factors influencing, V, 284, 289 ff.
manganese and, IV, 218-221, 238
mechanism of, IV, 235-239
inactivation of, V, 293
inhibition of goitrogenic agents by, V, 276, 278
from iodinated proteins, IV, 213, 214, 220, 222
iodine and, IV, 121
pituitary and, V, 280
potency of optical isomers of, IV, 230-232
sulfaguanidine and, III, 63
thiourea and, V, 286
Tibia,
effect of growth hormone on, V, 200
Tissues,
cancerous,
acid phosphatase and, II, 355
inositol and, II, 321
oxidation capacity of, II, 337
vitamins in, II, 319, 320, 329
vitamins and, III, 1, 8-11, 23
Toad poisons,
crystal structure of, II, 441, 442, 445
 α -Tocopherol (vitamin E), I, 60; II, 114, 120; III, 58, 69
absorption of, I, 162
absorption spectra of, I, 117, 134-137
activity of, II, 119, 120
antisterility action of, II, 118
assay of, I, 138
creatine metabolism and, I, 180
derivatives of, II, 119, 127, 134
distribution of, I, 137, 231, 232, 236; II, 116-118
international standard of, II, 114
isolation of, II, 108
treatment of amyotrophic lateral sclerosis with, I, 96, 97
vitamin A deficiency and, II, 325
 β -Tocopherol,
activity of, II, 118-120
derivatives of, II, 119
 γ -Tocopherol,
antisterility potency of, II, 118
biological activity of, II, 119
dl- α -Tocopherolquinone,
effect on mice, V, 193
Tocopherols,
absorption spectrum of, II, 111
activity of, II, 119
as antioxidants, II, 144; III, 3, 14
antisterility action of, II, 145
carotene and, III, 14; V, 59
chemistry of, II, 107-109
covitamin action with palmityl ascorbic acid, III, 14
destruction by cod liver oil, III, 12
determination, II, 112; V, 44
effect on mice, V, 193
on muscular dystrophy, III, 12
growth and, III, 14
international standard, II, 119
interrelations with other vitamins, II, 144; III, 2-3, 12, 14, 15; V, 60
levels in sprue, V, 191
requirements of invertebrates, II, 116
of mice, V, 191
sources, II, 116-119; III, 14
Torpedo,
electric organ of, III, 352-355
Torula,
vitamin requirement of, III, 123, 182
Toxisterol, I, 134
Trauma,
vitamin K deficiency and, IV, 117
Trigonelline, I, 29, 160
niacin deficiency and, III, 29
urinary excretion of, IV, 96, 98

- Trimethyl- β -hydroxyethylammonium hydroxide, see Choline
- Triphenylethylene,
effect on plumage, II, 399
on testes, V, 240
- Triphosphopyridine nucleotide, see also Coenzyme II, III, 7
effect on yellow enzyme, III, 6
niacinamide in, III, 6
- Trypanosoma lewisi*,
biotin deficiency and resistance to, IV, 56
- Trypanosomiasis,
vitamin B complex deficiency and susceptibility to, IV, 46
- Trypsin, I, 162, 286
- Tryptophol, I, 159
- Tryptophan, I, 159, 182
as accessory factor to antianemic substance, III, 281-283
ariboflavinosis and, IV, 94
niacin requirement and, V, 81
- Tumors, II, 323, 343
adrenal, I, 309, 329
urinary excretion of androgens in persons with, I, 309-311
of estrogens, I, 304, 329
choline and, I, 40
competitive vitamins and growth of malignant, II, 342
effect of coenzymes on, II, 319
of nicotine on, II, 319
of unsaturated fatty acids on, II, 21
of yeast on, II, 332
endocrines and malignant, II, 353
estrogens and, V, 248
growth of, II, 344
hepatic, see also Hepatomas, I, 39
hormones in, II, 357
induced by benzopyrene, II, 326
by p-dimethylaminoazobenzene, II, 305, 320-326, 331-341
methylcholanthrene, II, 325
by ultraviolet light, II, 325
mammary, II, 353
metabolism and, II, 305
nutrition and malignant, II, 339
resulting from embryonal development, III, 98
spontaneous, II, 323
testis,
gonadotropic substances and, II, 353
urinalysis in the diagnosis of, II, 357
thiouracil and, V, 279
thiourea and, V, 306, 315
thyroid, V, 278, 279
arising from treatment with anti-thyroid substances, V, 306, 315
vitamins and, I, 246; II, 33, 36, 142, 305, 306, 309-318, 320, 322-328, 339, 344, 345
- Tungstic acid,
effect on resorption of gonadotropin, III, 323
- Turkey,
vitamin requirement of, V, 169 ff
- Tyrosinase, I, 171, 324
inhibitory effects of goitrogenic agents on, V, 292
- Tyrosine, I, 19, 159, 176, 183, 184
as accessory factor to antipernicious anemia substance, III, 265, 274, 276, 287
alcaptonuria and, I, 185
p-aminobenzoic acid and, I, 171; II, 231
ascorbic acid and, I, 187; V, 81
in casein, IV, 220
combination with iodine, IV, 210, 211, 214, 216
dietary protein and requirement for, IV, 101
dissociation constant of iodinated, IV, 240
hemopoietic properties of, IV, 26
- Tyrosol, I, 159
- Tyrosine, as bacteriostatic agent, II, 345
- U
- Ulcers,
choline in, II, 321
vitamin deficiency and, II, 72-74, 320
- Undernutrition, IV, 74, 75
anestrus and, IV, 144
caloric intake and, IV, 74
effect on reproduction, IV, 74
in infancy, IV, 74
manifestations of, IV, 74, 75
protein deficiency and, IV, 76, 77
resistance to bacterial infection and, IV, 49
use of testosterone propionate in, IV, 268-270
- Uracil,
as bacterial growth factor, III, 140, 198-201
bacterial synthesis of, III, 198
- 3(β),11-Uranediol, I, 306
- Uranetriol, see Pregnanetriol A
- Uranol-11-one-3, I, 306

- Urea, I, 186, 347
 effect of steroids on, in blood and urine, IV, 277-287
- Urease, II, 339
 p-aminobenzoic acid and, II, 244
 inhibition by xanthine oxidase, II, 339
- Uric acid, I, 129
- Urine, I, 83, 98, 179, 184, 185; II, 355
 p-aminobenzoic acid in, II, 241
 androgenic activity of, I, 308, 311
 bile pigments in, I, 279
 output, I, 175
 of pregnancy,
 estrogens in, I, 305, 327, 328, 335;
 II, 385
 pregnanediol in, I, 300, 301, 304
 reduction products of progesterone
 in, I, 297, 298, 304, 313
 vitamin excretion in, III, 29, 39, 41,
 42, 45, 53, 84; IV, 27, 86, 100
- Urobilinogen, I, 279
- Uropterine, IV, 26
- Ustilago,
 thiamine requirements of, III, 123
- Uterus, I, 232, 302, 305; IV, 162, 163; V,
 355
 effect of androgens on, II, 372; V, 329-
 331
 functional bleeding, IV, 152, 154, 168
 therapeutic effect of androgens in,
 V, 339-345
 masculinus, effect of estrogens on, V,
 255, 257
 vitamins and, II, 116, 117, 122, 135,
 143; IV, 162
 weight of,
 estrogens and, IV, 138
 liver and, IV, 138
- V
- "V" factor, III, 110, 186-191
 bacterial assay of, III, 188
 identity with coenzymes I and II, III,
 186
 specificity of, III, 188
 synthesis of, III, 187, 189
- Vagina,
 effect of androgens on, II, 373; V, 331-
 333
 of estrogens on, IV, 138, 323, 324
 manifestations of vitamin A deficiency
 in, II, 373; III, 80, 81
- Vagus,
 action of ultraviolet light on, perfusion
 fluid, V, 112
 liberation of thiamine on stimulation
 of, V, 111
 "Vagusstoff," III, 338; V, 114
 effect of atropine on, III, 338
- Valeric acid, II, 60 ff.
- biotin and, II, 48, 62
- Valine, I, 19
- Vasa deferentia,
 development of, II, 365
 effect of androsterone on, II, 376
- Vascular system, II, 122, 123
 dysfunction of, II, 67, 137
 vitamin E and, II, 144
 vitamin K and, II, 144
- Ventriculin,
 pellagra and, IV, 95
- Viosterol,
 rickets and, II, 76
- Virilism,
 androgens and, I, 308, 311; V, 336
- Virus,
 action of sulfonamides on, III, 177
 vitamins and resistance to, IV, 42, 48,
 57
- Vision, I, 223
 vitamins and, I, 88, 89, 197, 224, 22
 IV, 83
- Visual purple, see also Rhodopsin
 vitamins and, III, 4, 5, 6, 17
- Visual yellow, see Retinene
- Vitagen, I, 50
- Vitamin A, I, 134, 195, 196, 215, 238,
 239; IV, 50-53, 79-86
 absorption, chemical, I, 163
 gastrointestinal, II, 204; IV, 80; V,
 31
 absorption spectra of, II, 157, 158, 166,
 174, 175, 192, 193
 of precursors of, I, 134
 activity of, II, 162, 165, 171, 183, 197;
 II, 78
 assay of, I, 133, 145, 151; II, 157-159,
 189, 191, 198, 201
 benzopyrene and, II, 310, 311
 bixin and, II, 162
 bone formation and, III, 9, 10
 calcium metabolism and, II, 330
 carotene and, II, 161, 183, 195, 197,
 202; III, 82; IV, 79
 chemistry of, II, 155, 174
 configuration of, V, 32
 conversion of dimethylaminovitamin
 A to, V, 25-27
 cyclized, I, 134
 deficiency, I, 100, 164, 225; II, 71, 72,
 74, 75, 82, 95, 102, 199, 311

- effect of maternal, on fetus, III, 81-83, 97, 98
- immunological aspects of, IV, 63
- manifestations of, I, 83, 84, 88, 99, 100, 164; II, 189, 190-192, 198-200, 202, 204, 284, 309, 311, 325; 330; III, 10, 13, 80-84, 98; IV, 82-86
- dental caries and, I, 63
- derivatives of, II, 167, 171-173, 177, 178
- determination of, IV, 80; V, 42
- effect on colostrum, II, 194, 195
- immunological reactions, I, 173
- excretion of, II, 205; IV, 80
- fat and, II, 7; IV, 79
- fucoxanthin and, II, 162
- as growth factor, II, 189, 198, 222, 311; III, 14
- homogeneity of, V, 33
- inactivation of, II, 159
- international unit of, II, 181, 189
- interrelations with other vitamins, II, 73, 91, 145, 187, 192, 193, 199, 202; III, 1-5, 8, 9, 12-17
- isolation of, II, 155
- isomers of, II, 178
- lycopene and, II, 162
- occurrence, I, 98, 177, 218, 220, 230, 231, 233, 235-237, 239-241; II, 156-167, 169, 171, 173, 192, 195-199, 305, 309, 310, 336; III, 16, 82; IV, 51, 79-82
- neovitamin A and, V, 33
- physiology of, I, 239; II, 155; IV, 79-81
- placental passage of, II, 198
- photodegradation products of, III, 5
- porphyropsin and, II, 202
- preparations of, II, 171, 204
- provitamin A and, II, 155; IV, 79
- requirements, I, 71, 75, 76, 82; II, 203
- of chicks, V, 164, 165, 170, 171
- of mice, V, 190
- pituitary and, IV, 144
- during pregnancy, III, 96
- resistance to bacterial infection and, II, 199, 200; IV, 50, 52, 53, 56, 57
- stability of, II, 169, 170, 204
- stereochemistry of, V, 30
- stereoisomers of, V, 1-4
- synthesis, II, 155, 178, 184; V, 1-4
- toxic effects of, II, 204; III, 15-17
- treatment of hypervitaminosis D with, III, 17
- tumors and, II, 31, 195-198, 295, 305, 309-312, 325, 326, 329, 330, 334
- vision and, I, 167, 186, 195, 197, 214, 217, 219, 220, 222, 223, 225; III, 4-6, 17; IV, 83
- visual purple and, III, 4, 17; IV, 83
- Vitamin A₁,
- absorption spectrum of, I, 117, 132
- assay of, I, 138
- determination of, I, 144
- distribution of, I, 133, 220
- iodopsin and, I, 223
- retina and, I, 195, 219
- vision and, I, 195, 215-217, 219, 221, 222, 224
- vitamin A₂ and, I, 218, 221
- Vitamin A₂,
- absorption spectrum of, I, 117, 132
- determination of, I, 144
- distribution of, I, 222, 230; II, 187, 188
- lycopene and, II, 188
- porphyropsin and, I, 167, 215, 216, 220
- retina and, I, 186, 221, 222
- rhodopsin, II, 202
- structure of, II, 188
- vision and, I, 167, 186, 215, 216, 218, 221-224
- vitamin A₁ and, I, 218, 221
- Vitamin A₃,
- absorption spectrum of, I, 117, 132
- determination of, I, 144
- "Vitamin A acid," V, 27-30
- Vitamin A aldehyde,
- identity with retinene, III, 6
- preparation from vitamin A, III, 6
- Vitamin A esters, I, 71, 240
- synthesis of, V, 22-25
- Vitamin A ethers, V, 10-22
- biological activity of, V, 13, 14
- synthesis, V, 9-22
- ultraviolet absorption spectra of, V, 20
- Vitamin A homologues,
- biological activity of, V, 4
- synthesis of, V, 4-9
- ultraviolet absorption spectra, V, 9
- Vitamin B₁, see Thiamine
- Vitamin B₂, see Riboflavin
- Vitamin B₆, see also Pyridoxine
- carbohydrates and requirement of, V, 73
- and metabolism of unsaturated fatty acids, V, 73
- response of yeast to, V, 46, 72
- Vitamin B₁₀, IV, 29
- feather development and, IV, 15
- folic acid and, IV, 15, 16
- hematopoiesis and, IV, 2
- properties of, IV, 15

- α -pyracin and, IV, 16
- pyridoxal and, IV, 16
- pyridoxamine and, IV, 16
- vitamin B₆ and, IV, 12, 16
- Vitamin B₁₁, IV, 30
- folic acid and, IV, 15, 16
- hematopoiesis and, IV, 2
- properties of, IV, 15
- α -pyracin and, IV, 16
- pyridoxal and, IV, 16
- pyridoxamine and, IV, 16
- vitamin B₆ and, IV, 12, 16
- Vitamin B₆, see also Folic acid, *L. casei*
 - factor, IV, 6, 8-11, 14, 29; V, 120, 121
 - anemia and, III, 62; IV, 8, 13, 24
 - assay of, IV, 16
 - deficiency,
 - manifestations of, IV, 10
 - xanthopterine and, IV, 26
 - factor R and, IV, 13
 - factor S and, IV, 13
 - folic acid and, III, 59, 61
 - granulocytopenia and, IV, 23
 - hematopoiesis and, IV, 2
 - L. casei* factor and, III, 60; IV, 6, 29
 - leucopenia and, IV, 23
 - liver and, IV, 13
 - norite eluate factor and, IV, 9
 - optimum requirement of, IV, 10
 - properties of, IV, 10
 - vitamin B₆ conjugate and, IV, 11-13
 - vitamin B₁₀ and, IV, 12, 16
 - vitamin B₁₁ and, IV, 12, 16
 - vitamin M and, IV, 12
 - xanthopterine and, IV, 25-27
 - in yeast, IV, 12
- Vitamin B₆ conjugase, IV, 13-15
 - distribution of, IV, 13, 14
 - properties of, IV, 13, 14
 - S. lactis* R factor and, IV, 13
 - vitamin B₆ conjugate and, IV, 13-15
- Vitamin B₆ conjugate, IV, 11-13, 29; V, 120
 - factor R and, IV, 18
 - factor U and, IV, 18
 - hematopoiesis and, IV, 2
 - L. casei* growth and, IV, 11
 - in liver, IV, 12
 - in milk, IV, 25
 - phosphatase and, IV, 14
 - properties of, IV, 12
 - response to pernicious anemia, V, 151
 - S. lactis* R growth and, IV, 11
 - succinylsulfathiazole and, IV, 23
 - vitamin B₆ conjugase and, IV, 13-15
 - vitamin M and, IV, 21
 - from yeast, IV, 12
- Vitamin B complex, I, 60
 - cellular metabolism and, I, 244
 - cholesterol formation and, II, 14
 - choline and, I, 51; II, 6, 12
 - concentrate, I, 92
 - deficiency, I, 87, 161, 164, 224; IV, 20
 - breeding and, III, 77
 - estrogens and, IV, 137-139, 144
 - eye lens and, III, 8
 - gonadal hormones and, IV, 136
 - gonadotropic hormones and, IV, 136
 - growth and, III, 27
 - immunological aspects of, IV, 63
 - ovarian function and, IV, 137
 - resistance to bacterial infection and, IV, 45, 46, 53, 55-57
 - in diet, I, 39, 42; II, 9, 87; III, 94, 95
 - effect of clarase and papain on, I, 234
 - enzymes and, I, 137, 245
 - estrogen metabolism and, IV, 137-140
 - extrinsic factor and, IV, 1
 - fat synthesis and, II, 4, 6, 8
 - as growth factor, I, 246, 255, 256, 259
 - interrelations between components of, I, 235; II, 3; III, 33
 - with other vitamins, II, 29; III, 8
 - intestinal synthesis of, III, 23-48
 - lactation and, IV, 140, 141
 - liver and, IV, 138
 - occurrence, I, 98, 233, 234, 236, 283; II, 3, 13
 - reproduction and, IV, 135-144
 - requirement, I, 177
 - dietary protein and, III, 7, 8
 - resistance to infection and, I, 172
 - therapeutic use of, I, 90, 97; III, 16, 17; IV, 154, 161, 162, 179
 - tumors and, II, 312, 322, 329, 330, 344
 - unknown factors in, I, 94
 - yeast and, II, 83
- Vitamin C, see Ascorbic acid
- Vitamin D, I, 59, 60; IV, 107-113
 - absorption spectra of, I, 134
 - action of, II, 79
 - bones and, III, 9; IV, 41, 109
 - calcium and, I, 158; III, 18; IV, 107-110
 - deficiency, I, 77, 162, 164; II, 75-77, 284
 - immunological aspects of, IV, 63, 64
 - manifestations of, III, 89; IV, 109, 111-113
 - determination of, V, 44
 - dibenzanthracene and, II, 310
 - dietary carotene and, II, 81

- effect on asthma, I, 174
 - on hay fever, I, 174
 - on phosphorus metabolism, I, 158
 - on reproduction, III, 92, 94, 95
 - on serum phosphatase, I, 171; IV, 110
 - on teeth, I, 63; III, 9
- ergosterol and, II, 78
- as growth factor, I, 261; II, 222
- hair and, IV, 41
- hemoglobin regeneration and, I, 175
- interrelations with other vitamins, II, 91; III, 15, 16
- intestinal absorption of, I, 162
- occurrence, I, 79, 230, 231; II, 156, 310; IV, 108, 109
- photochemistry and, I, 110
- placenta and, IV, 108
- requirements, I, 75, 82; III, 11, 15, 18; IV, 109; V, 164-166, 169-171, 190
- resistance to bacterial infection and, IV, 50, 57
- rickets, I, 95; IV, 107
- tetany and, IV, 113
- therapeutic use of, III, 15, 16
- tumors and, II, 324, 325, 334
- Vitamin D₂, I, 117, 138, 144
- Vitamin D₃, I, 117, 138, 144
- Vitamin D₄, I, 117
- Vitamin E, see also α -Tocopherol, III, 58; IV, 114
 - androgens and, II, 327
 - antiestrogenic properties of, II, 129
 - antioxidant activity of, II, 199
 - bacterial synthesis of, III, 68
 - bone formation and, III, 9
 - carotene and, III, 13
 - cholesterol and, II, 327
 - compound with inositol, III, 68
 - concentrate, from wheat germ oil, II, 108
 - deficiency, II, 114, 116, 327; III, 13
 - anterior pituitary and, III, 9
 - manifestations of, II, 107, 121, 122, 143, 199; III, 10, 67
 - destruction by marine oils, III, 12
 - effect on endocrines, II, 325, 327
 - on seminiferous epithelium, II, 122-125
 - fat synthesis and, III, 11, 12, 18
 - homologues of, II, 107
 - international unit of, II, 113, 114
 - interrelations with other vitamins, II, 107, 144, 145, 199; III, 1, 9, 12-15, 18
 - metabolism of, II, 325
 - muscular dystrophy and, III, 12; IV, 114; V, 168
 - physiology of, II, 107, 108, 113, 116, 117, 145
 - progesterone and, II, 327, reproduction and, III, 90, 91; IV, 136
 - requirement for, II, 107, 142, 143; V, 168, 190, 191
 - sources of, II, 115-117, 136
 - tumors and, II, 325-327, 334
- Vitamin H, see also Biotin
 - absorption of, II, 38
 - p-aminobenzoic acid and, II, 238
 - assay of, II, 32
 - concentrate of, II, 38-40
 - egg white injury and, II, 31
 - identity with biotin, III, 160, 169
- Vitamin K, I, 97, 103, 163; III, 91; IV, 114-119
 - absorption spectrum, I, 117, 137
 - analogs, I, 176; II, 342; V, 193
 - antagonism to p-aminobenzoic acid, III, 67
 - bile salts and absorption of, IV, 115
 - biosynthesis, II, 327; III, 35, 51, 52, 66-68; IV, 116
 - clotting of blood and, I, 97; IV, 58, 114-117
 - deficiency, I, 97, 176; II, 327
 - effect of pteroylglutamic acid on, V, 134
 - manifestations of, III, 32, 35, 52, 56, 67, 97; IV, 116-119
 - sulfonamides and, III, 32, 54, 56, 66
 - determination of, I, 144
 - distribution of, I, 232; II, 115; IV, 115, 116
 - effect on enzymes, II, 327
 - on experimental cancer, II, 327
 - interrelations with other vitamins, II, 112, 144; III, 32
 - intestinal absorption of, I, 162
 - intrinsic liver disease and, I, 97
 - oxidation-reduction system and, II, 327
 - prothrombin and, I, 103, 157, 176, 186; II, 327
 - requirement for IV, 116; V, 167, 171
 - resistance to infection and, IV, 50
- Vitamin K₁,
 - absorption spectrum of, I, 117, 136, 137
 - assay of, I, 138
- Vitamin K₂,
 - absorption spectra of, I, 117, 136, 137
- Vitamin K-active substances, III, 214, 216

- bacterial synthesis of, III, 274
- Vitamin M,
 anemia and, IV, 19, 20; V, 140, 151
 blood cells and, IV, 19
 deficiency, V, 125
 cytopenia and, III, 61; IV, 57
 L. casei factor and, III, 61
 manifestations, II, 73; IV, 57
 sprue and, V, 125
 edema and, IV, 19
 folic acid and, IV, 12, 21
 hemoglobin and, IV, 19
 hematopoiesis and, IV, 2
 isolation, II, 99
 nutritional importance of, V, 139, 155 ff.
 requirements, V, 136 ff.
 resistance to bacterial infection and,
 II, 97, 98; IV, 56, 57
 sources of, II, 73, 100; V, 155
 xanthopterin and, IV, 25, 26
- Vitamin P, I, 97; V, 62
 effect on ascorbic requirement, III, 4
 on capillaries, I, 97; III, 4
- Vitamin PP, see Niacin
- Vitamin analogues,
 effect on mice, V, 191-193
- Vitamin(s),
 absorption spectra of, I, 109, 115-117,
 127, 130
 amino acids and, I, 157-194; V, 41
 analogues of, II, 341, 342
 assay of, I, 112, 138, 142, 143, 151,
 165, 223; V, 47-49
 availability of, III, 35, 50; V, 54, 82, 83
 bile acids and absorption of, I, 164
 biosynthesis of, I, 157, 159; III, 23,
 50, 66; V, 50, 80 ff.
 as catalysts, I, 245
 cancer and, II, 305-359
 choline as, I, 160
 concentrate from milk, I, 180
 deficiency, I, 163, 187; V, 124
 antivitamins and, V, 71
 manifestations of, III, 10
 prenatal development and, III, 80-
 91
 determination of, I, 44
 in diet, I, 60, 75, 163, 173; IV, 144
 endocrine glands and, II, 345
 enzymes and, I, 157, 170, 171; II,
 306, 337, 345
 fat synthesis and, III, 11, 12, 42
 as growth factor for protozoa, I, 249,
 250
 hemoglobin metabolism and, I, 157,
 186
 immunity and, I, 157, 172, 173
 inhibitors of, III, 99
 interrelations with hormones, IV, 137,
 143, 144, 149 ff.
 between, III, 2; V, 40, 71, 72, 81 ff.
 proteins and, I, 157-194
 requirements, III, 1, 18, 42; V, 164-
 171, 175-194
 in sweat, V, 50
 in tissues, I, 229
 toxic effects of, III, 15
 urinary excretion, V, 50, 52
- Vocal cords,
 effect of androgens on, V, 336
- Vulva,
 effect of androgens on, V, 335
- ### W
- Water,
 deficiency, IV, 78, 79
 requirement of, IV, 78
 sources of, IV, 78
- Weight,
 effect of androgens on, V, 267
 of estrogens on, V, 267
 of growth hormone on, V, 212
 of protein deficiency on, IV, 77
 of vitamin A deficiency on, IV, 84
 urinary nitrogen and, V, 212
- Wheat germ oil,
 tumors and, II, 325, 326, 334
 use of, II, 128
 vitamin E in, II, 115
- Wounds, healing of,
 ascorbic acid and, I, 95, 169; IV, 106
 protein and, IV, 106
- ### X
- "X" factor, III, 110, 186-188
- X-zone,
 effects of estrogens on, V, 262
- Xanthine, I, 39
 as accessory factor to antianemic sub-
 stance, III, 267
 nutrient for lactic acid bacteria, III,
 200
- p-Xanthine,
 antithyroid activity of, V, 305
- Xanthine oxidase, I, 166
 effect of goitrogenic agents on, V, 291
 inactivation of, II, 339
 liver and, II, 317, 336
 nucleoprotein metabolism and, II,
 317

- vitamins and, II, 315, 337
- Xanthophyll**,
 absorption spectra of, I, 204, 205, 207
 carotene, I, 198
 distribution of, I, 196, 197, 199, 208,
 223; II, 194
 growth activity of, II, 161, 167
 in plant photokinesis, I, 223, 224
- Xanthopterine**, IV, 25-27
 activity of, V, 76
 blood and, IV, 26
 folic acid and, IV, 25, 27
 growth and, IV, 26
 properties of, IV, 25, 26
 reticulocyte response and, IV, 26
S. lactis R factor and, IV, 27
 succinylsulfathiazole and, IV, 26
 uropterine and, IV, 26
 vitamin B₆ and, IV, 25-27
 vitamin M and, II, 99; IV, 25, 26
- Xanthurenic acid**,
 excretion in mice, V, 187
- Xerophthalmia**,
 and epithelial metaplasia, II, 200
 in vitamin A deficiency, II, 74, 189,
 190, 200

Y

- Yeast**, I, 35, 36, 38, 229, 320, 321
 p-aminobenzoic acid and, II, 243
 as amphicarcinogen, II, 344
 choline and, II, 6
 cocarboxylase and, II, 16
 effect on lactation, IV, 141
 on liver, IV, 2

- on reproduction, IV, 136
 estrus cycle and, IV, 136
 extrinsic factor in, I, 286
 fat synthesis and, II, 4, 15-17, 23
 as growth factor for protozoa, I, 263
 hepatic tumors and, I, 39
 I₁ factor in, IV, 140
 I₂ factor in, IV, 140
 pernicious anemia and, I, 284, 287
 steroid hormones and, I, 295, 320-322
 sterols in, I, 231
 synthesis of thiazole by, I, 159
 vitamins and, I, 100, 168, 230, 232,
 234, 244; II, 33, 37, 99, 315, 318;
 IV, 13, 91
- Yeasts**, III, 63, 77
 β-alanine requirement of, III, 152
 cocarboxylase in, III, 6
 folic acid in, III, 59
 inositol requirement of, III, 207, 208
 osmophilic, III, 207
 symbiosis with molds, III, 33
 therapeutic use of, III, 15, 16
 vitamin requirements of, III, 123, 141,
 143, 157-162
- Yellow enzyme**, I, 166

Z

- Zeaxanthine dipalmitate**, I, 199
- Zinc salts**,
 effect on resorption of gonadotropin,
 III, 323
- Zymogen**, I, 285
- Zymosterol**,
 crystal structure of, II, 454, 455

Author Index

Names in parentheses indicate senior authors of the references and are included to assist in locating references where a particular name is not on a given page. The numbers in parentheses are reference numbers; those in italics refer to the pages on which references are listed in bibliographies at the end of each article.

Examples: Abbott, W. E., 284 (see Mellors) means that Mellors *et al.* will be mentioned on page 284, the *et al.* accounting for Abbott. This article can be located under Mellors in the list of references.

Abdel-Salaam, A., 166 (ref. 1) means that reference number 1 is mentioned on page 166, without the author's name.

A

- Abbasy, M. A., 74, 86, 93
 Abbott, W. E., 284 (see Mellors), 325
 Abdel-Salaam, A., 166 (ref. 1), 168, 214
 Abderhalden, E., 77, 93
 Abderhalden, R., 77, 93
 Abelin, I., 118, 157
 Abels, J. C., 83, 98
 Abt, A. F., 86, 87 (see Farmer), 93, 95
 Adams, G. A., 211 (ref. 2), 212, 214
 Adams, M., 317 (see Wilder), 327
 Adamson, J. D., 141, 143, 145, 147, 157
 Adcock, E. W., 136, 157
 Addicott, F. T., 227, 228, 229, 236, 270, 271
 Adolph, W. H., 122, 157
 Agarwal, G. N., 166 (ref. 272), 174 (ref. 272), 178 (see Singh), 184 (ref. 272), 187 (see Singh), 189 (ref. 272), 190 (see Singh), 199 (see Singh), 221
 Agarwal, P. N., 166 (ref. 3), 174 (ref. 3), 184 (ref. 3), 198, 199, 214
 Aggeler, P. M., 71, 97
 Aguilar, E., 245 (see Cravioto), 246 (see Cravioto), 272
 Ahlgren, G. H., 257, 258, 270
 Albanese, A. A., 146, 150 (see Holt), 157, 158
 Albaum, H. G., 265, 270
 Alcock, A. W., 252 (see Hoffer), 253, 254, 272
 Alcott, D. L., 311, 321
 Alexander, W., 71, 96
 Alexopoulos, C. J., 167, 217
 Alington, B. K., 115, 161
 Allen, R. J. L., 202, 215
 Allers, W. D., 280, 285, 305 (see Kendall), 310, 321, 324
 Allinson, M. J. C., 188, 214
 Almquist, H. J., 28, 30, 48, 207, 208, 214
 Alscher, R. P., 42 (see Light), 51, 89 (see Light), 97
 Amberg, E. J., 87 (see Holmes), 96
 Amberg, S., 304, 321
 Anderson, C. R., 82 (see Snyder), 99
 Anderson, E. P., 56, 60, 66
 Anderson, Evelyn, 293, 294, 296, 297, 299, 312, 321
 Anderson, R. C., 5 (see Wolf), 12 (see Wolf), 25, 202 (see Wolf), 223
 Anderson, R. J., 204, 207, 214
 Anderson, R. K., 245 (see Cravioto), 246 (see Cravioto), 272
 Anderson, W. E., 281 (see Light), 324
 Andrus, W. D., 45, 51, 72, 97
 Anfinssen, Chr. B., 36 (see Ball), 48
 Angerer, C. A., 285, 321
 Angerer, H., 285, 321
 Angier, R. B., 6, 7 (see Stokstad), 8 (see Mowat; Stokstad), 9 (see Hutchings; Stokstad), 11 (see Mowat), 13, 14 (see Waller), 15 (see Boothe; Hutchings; Mowat; Waller), 16 (see Hutchings; Waller), 19, 21 (see Hutchings), 22, 23, 24, 25, 85, 93, 198, 201 (see Waller), 214, 217, 223
 Anigstein, L., 82, 93
 Ansbacher, S., 30 (see Fernholz), 49, 93, 94, 139, 157, 191, 214
 Antopol, W., 79, 80, 94
 Archdeacon, J. W., 127, 128, 157
 Argenziano, R., 31, 50
 Armentano, L., 88, 94

- Arminio, J., 122 (see Wald), 161
 Armstrong, C., 56, 66
 Armstrong, C. W. J., 311 (see Cleghorn), 322
 Armstrong, M. R., 29, 48
 Armstrong, W. D., 36, 46, 48, 93, 94, 144, 159
 Arnold, A., 34, 49
 Arnold, F. A., 62, 66
 Arnold, W. T., 82, 97
 Arnon, D. I., 255, 257, 258, 270
 Aron, H. C. S., 87 (see Farmer), 95
 Arth, G. E., 5 (see Wolf), 12 (see Wolf), 25, 202 (see Wolf), 223
 Arzberger, C. F., 175, 176, 214
 Ascher, K. W., 145, 146, 161
 Ashford, C. A., 87, 100
 Ashida, K., 171, 214
 Ashworth, C. T., 284, 321
 Asper, S. P., 77 (see Williams, R. H.), 100
 Astwood, E. B., 313 (see Talbot), 327
 Atchley, D. W., 280 (see Loeb), 281 (see Loeb), 287 (see Loeb), 289 (see Loeb), 314 (see Kuhlmann; Ragan), 315 (see Ferrebee; Kuhlmann), 316 (see Loeb), 319 (see Ferrebee), 323, 324, 325
 Atkin, L., 170 (see Schultz, A. S.) 171 (see Schultz, A. S.), 172 (see Schultz, A. S.), 183 (see Schultz, A. S.), 195 (ref. 15), 214, 221
 Atkins, P., 36, 98
 Atkinson, M., 78, 94
 Ausherman, L. E., 238 (see Brink), 271
 Austen, D. C., 311 (see Cleghorn), 322
 Avakian, S., 17, 23
 Avery, G. S. Jr., 239, 263, 270, 271
 Axelrod, A. E., 80, 90 (see Becks), 94, 191, 214, 217, 223
 Axelrod, H. E., 90 (see Morgan), 97
 Axtman, G., 238, 271
- B**
- Bacharach, A. L., 88, 94, 125, 157
 Bacher, F. A., 32, 33, 53
 Bader, M. N., 82, 93
 Baetjer, A. M., 283, 321
 Baird, D., 132, 157
 Baird, G. R., 188, 217
 Baker, B. R., 28, 48
 Baldwin, I. L., 166 (ref. 177, 322), 169 (see VanLanen), 170 (see VanLanen), 171 (see Pavcek; VanLanen), 172 (see VanLanen), 173 (see VanLanen), 182, 184 (ref. 177), 190, 192 (see Lampen), 193 (see Lampen), 217, 219, 220, 223
 Bale, W. F., 282, 325
 Balfour, M. I., 131, 157
 Ball, E. G., 36, 48
 Ballentino, F., 183 (see Ryan), 191 (see Ryan), 221
 Banting, F. G., 279, 280, 289, 294, 321
 Bantz, A. C., 174 (ref. 319), 183 (see Underkoffler), 222, 265 (see Underkoffler), 275
 Barborka, C. J., 127, 157, 158
 Barcroft, H., 87, 94
 Barker, D. E., 291 (see Visscher), 327
 Barker, H. A., 174 (ref. 18), 175, 200, 214
 Barlow, O. W., 87 (see McChesney), 97
 Barnes, R. H., 296, 301, 304, 321, 322, 325
 Barrett, R., 165, 184 (ref. 199), 219
 Bartlett, G., 261, 270
 Bartlett, M. K., 87, 94
 Bartley, M. A., 227, 228, 230, 231, 274
 Bauernfeind, J. C., 210, 211 (ref. 19, 20), 214
 Baumann, C. A., 209, 214, 217
 Baumann, E. J., 279, 281, 321, 325
 Baumberger, J. P., 33, 48
 Baumgarten, W., 210 (see Bauernfeind), 211 (ref. 19, 20), 214
 Bavetta, L. A., 296, 321
 Bazavan, G., 71 (see Hagiesco), 95
 Beach, E. F., 120, 160
 Beadle, G. W., 185, 186, 193, 197, 215, 222, 263, 265, 270, 274
 Bean, W. B., 78 (see Spies), 99
 Beck, A. C., 44, 49
 Beck, R. D., 91 (see Kaufman), 96
 Becker, E. R., 81, 99
 Becks, H., 90, 91, 94
 deBecze, G., 180, 219
 Beesch, S. C., 176, 218
 Begtrup, H., 44, 45, 49, 50
 Bell, M., 131 (see Ebbs), 158
 Bell, T. T., 233, 261, 274

- Bellamy, W. D., 195, 196, *215*, 264, *272*
 Belt, M., 16 (see Franklin), *23*
 Bencsik, J., 128, *158*
 Bendich, A., 33, *49*
 Benedict, E. M., 280 (see Loeb), 281
 (see Loeb), 287 (see Loeb), 289 (see
 Loeb), *325*
 Benesch, R., 116, *157*
 Benko, A., 88, *98*
 Bercovitz, Z., 44, *49*, 70, *98*
 Berger, J., 263, *270*
 Bergman, H. C., 312 (see MacKay,
 E. M.), *325*
 Bergmann, M., 15, *22*
 Berkman, S., 181 (see Dorfman), *216*
 Berlin, H., 31, *49*
 Bernhauer, K., 203, *215*
 Bernheim, F., 33, *49*
 Bernheim, M., 33, *49*
 Berryman, G. H., 127 (see Cogswell), *158*
 Bersin, T., 87 (see Lauber), *96*
 Bessey, O. A., 73, *97*, *100*, 108, 117, 121
 (see Lowry), 127 (see Kruse), *157*,
 159, *161*
 Best, C. H., 86, *94*
 Bicking, M. M., *218*
 Bicknell, F., 76, *94*
 Bierbaum, O. S., 85 (see Moore, C. V.), *97*
 Bills, C. E., 204, 205 (ref. 26), 206, *215*,
 219, *220*
 Bina, A. F., 195 (ref. 27), *215*
 Binger, M. H., 288, 310, *324*
 Bingham, A. W., 77, *94*
 Binkley, S. B., 1 (see Piffner), 2 (see
 Piffner), 3 (see Piffner), *24*, 28
 (see Doisy), *49*, 84 (see Piffner), 85
 (see Piffner), *98*, 198 (see Piffner),
 208 (see McKee; Thayer), *219*, *220*,
 222
 Bird, O. D., 1 (see Piffner), 2 (see
 Piffner), 7 (see Piffner), 19 (see
 Piffner), 20, 21 (see Mims), *22*, *23*,
 24, 84 (see Piffner), 85 (see Piffner),
 98, 174 (ref. 28), 186, 200, *215*
 Birkinshaw, J. H., 205 (ref. 29), *215*
 Black, S., 74, 81, *94*
 Blair, H. L., 123 (see Steffens), *161*
 Blakeslee, A. F., 238 (see Van Overbeek),
 275
 Blanchard, K. C., 192, *215*
 Blanchard, M., 21 (see Ratner), *24*, 193
 (see Ratner), 198 (see Ratner), *220*
 Bloom, E., 212 (see Sullivan), *222*
 Bloom, E. S., 1 (see Piffner), 2 (see
 Piffner), 3 (see Piffner), 6 (see
 O'Dell), 7 (see Piffner), 19 (see
 Piffner), 20 (see Piffner), *24*, 84
 (see Piffner), 85 (see Piffner), *98*,
 198 (see Piffner), 201 (see Piffner),
 220
 Bloomfield, R., 127 (see Egaña), *158*
 Bloor, W. R., 283 (see Fenn), *323*
 Blumberg, H., 34, *49*
 Boak, R. A., 283 (see Fenn), *323*
 Boas-Fixen, M. A., 203, *215*
 Bohonos, N., 4, *23*, 194, 201 (see Hutch-
 ings), *215*, *217*, 235, *271*
 Boissevain, C. H., 174 (ref. 33), 178, *215*
 Bollman, J. L., 283 (see Flock), 293
 (see Kendall), *323*, *324*
 Bonner, D., 185, 186, *215*, 241, *275*
 Bonner, D. M., 238, 240, 255, *270*
 Bonner, H., 173
 Bonner, J., 170, 173, *215*, 227, 228, 229,
 230, 231, 232, 233, 234, 235, 237, 238,
 244, 248, 251, 252, 254, 255, 256,
 257 (see Parker), 258, 259, 261, 262,
 266, 268, *270*, *271*, *274*, *275*
 Bonnet, R., 206 (see Terroine), *222*
 Booher, L. E., 122, *157*
 Boothe, J. H., 6, 7 (see Stokstad), 8, 9
 (see Hutchings; Stokstad), 11, 13, 14
 (see Waller), 15, 16 (see Hutchings;
 Waller), 19, 21, *22*, *23*, *24*, *25*, 85
 (see Angier), *93*, 198 (see Angier;
 Hutchings), 201 (see Waller), *214*,
 217, *223*
 Borchers, R., 181, *220*
 Borgström, G., 256, *271*
 von dem Borne, G. A. K., 319, *321*
 Borsook, H., 127, *157*
 Boruff, C. S., 210, 211 (ref. 19), *214*, *215*
 Bosecke, W., 29, 30, 31, *49*
 Bouillenne, R., 257, *271*
 Boulanger, P., 79, *96*
 Bourne, G., 202, *215*
 Bourne, G. H., 87, 88, *94*
 Bovarnick, M. R., 186, *215*
 Bowman, K. M., 72, 73 (see Jolliffe), *94*,
 96

- Braidotti, L., 30 (see Carrara), 49
 Bransby, E. R., 127, 157
 Brase, K. D., 258, 274
 Bratton, A. C., 7, 10, 21, 22
 Braunstein, A. E., 265, 271
 Brazda, F. G., 78, 94
 Brenner, M. W., 166 (ref. 266), 184 (ref. 266), 221
 Brenner, S., 123, 157
 Brewer, G., 283, 322
 Briggs, A. P., 140 (see Sydenstricker), 146 (see Sydenstricker), 161
 Briggs, G. M., Jr., 20, 22, 81, 94, 198 (see Mills), 199, 201 (see Mills), 218, 219
 Brink, R. A., 238, 271
 Brinkhous, K. M., 28, 49
 Britton, S. W., 280, 288, 298, 299, 300, 306, 322, 326
 Brock, M. J., 117 (see Bessey), 121 (see Lowry), 157, 159
 Brooke, R. O., 281, 322
 Broquist, H. P., 166 (ref. 322), 169 (see VanLanen), 170 (see VanLanen), 171 (see VanLanen), 172 (see VanLanen), 173 (see VanLanen), 223
 Brouha, L., 123 (see Wald), 127 (see Egafña; Johnson), 158, 161
 Broun, G. O., 62, 66
 Brown, A., 86, 95, 131 (see Ebbs), 158
 Brown, E. B., 195 (ref. 27), 215
 Brown, E. E., 35, 49
 Brown, G. B., 191 (see Dittmer), 219
 Brown, R. A., 1 (see Piffner), 2 (see Piffner), 7 (see Piffner), 19 (see Piffner), 24, 84 (see Piffner), 85 (see Piffner), 98
 Browne, J. A., 122, 157
 Brownell, K. A., 306, 322
 Brozek, J., 128, 129 (see Taylor), 157, 161
 Brozek, J. M., 127 (see Keys), 128 (see Keys), 159
 Bruce, W. F., 20 (see Scott), 24
 Brunius, E., 219
 Buchanan, J. M., 267 (see Sonne), 274
 Buchanan, R. N., 72 (see Turner), 99
 Buchman, E. R., 173, 215, 230, 232, 261, 262, 266, 271
 Buell, M. V., 285, 302, 322
 Büsing, K. H., 37, 52, 203, 215
 Buffon, 130
 Buhs, R. P., 31, 32, 34, 52
 Bunker, H. F., 166 (ref. 39), 184 (ref. 39), 215
 Bunker, J. W. M., 90 (see Harris, R. S.), 96
 Bunting, A. H., 261, 271
 Burgeff, H., 226, 259, 271
 Burchell, H. B., 309 (see Keith), 324
 Burgess, E., 77 (see Durand), 95
 Burk, D., 83 (see du Vigneaud), 95
 Burkholder, P. R., 167, 168, 179, 186, 200, 201, 212, 215, 242, 244, 248, 252, 253, 271
 Burns, H. S., 292, 322
 Burr, G. O., 296 (see Barnes), 301 (see Barnes), 321
 Burrill, D. Y., 47, 49
 Burris, R. H., 166 (ref. 149), 167, 168, 174 (ref. 149), 182 (ref. 149), 184 (ref. 149), 185, 189 (ref. 149), 218
 Buschke, W., 147, 157
 Bushey, M. S., 291 (see Visscher), 327
 Bushnell, L. D., 216
 Butler, A. M., 117, 157
 Butler, B., 194 (see Lyman), 218
 Butler, R. E., 70, 78, 99, 144, 160
 Butt, H. R., 28, 44 (see Clark, C. L.), 49, 82 (see Dry), 95
 Buxton, I. H. D., 131, 157

C

- Cagniant, P., 39 (see Meunier), 51
 Cain, C. K., 9, 17, 22, 23
 Calabresi, M., 308 (see Crismon, J. M.), 322
 Calandra, J. C., 46, 47 (see Burrill), 49, 50, 93, 94, 95
 Calder, R. M., 78, 94
 Caldwell, F. E., 83, 94
 Caldwell, W. A., 72, 94
 Calhoun, F. P., 122, 157
 Calkins, D. G., 2 (see Piffner), 7 (see Piffner), 19 (see Piffner), 20 (see Piffner), 24, 198 (see Piffner), 201 (see Piffner), 220
 Callison, E. C., 123 (see Booher), 157
 Callow, R. K., 205 (ref. 29), 215
 Calvert, L. S., 79, 94

- Calvin, D. B., 283 (see Mullin), 325
 Campbell, C. J., 2 (see Pfiffner), 7 (see Pfiffner), 19 (see Pfiffner), 24
 Campbell, H. A., 38, 41 (see Shapiro), 49, 53
 Campbell, K., 87 (see Holmes), 96
 Campbell, W. P., 30 (see Ficser), 50
 Campbell, W. W., 282 (see Greenberg), 323
 Carlson, G. H., 28, 48
 Carnes, W. H., 315 (see Ferrebee), 323
 Carpenter, C. C., 170, 215
 Carpenter, K. J., 116 (see Kodicek), 159
 Carr, C. W., 291 (see Visscher), 327
 Carrara, G., 30, 49
 Carter, B. B., 80 (see Axelrod), 94
 Caster, W. O., 121 (see Mickelsen), 160
 Castleden, L. I. M., 283, 322
 Cattell, McK., 283, 322
 Cavallito, C. J., 204, 205 (ref. 408), 215
 Cayer, D., 121, 127, 144, 157, 160
 Cerecedo, L. R., 166 (ref. 326), 170 (see Vinson), 223
 Chalet, L., 5 (see Rickes), 24
 Chargaff, E., 33, 49
 Chauchard, P., 125 (see Lecoq), 159
 Cheldelin, V. H., 166 (ref. 132), 169, 174 (ref. 132), 182 (ref. 132), 183, 184 (ref. 132), 189 (ref. 132), 190, 193 (ref. 132), 199, 215, 217, 243, 252, 265, 272
 Chen, G., 290, 322
 Chen, K. K., 40 (see Wakim), 53, 62 (see Rose), 66
 Christian, W., 226, 275
 Chu, Edith Ju Hwa, 28, 49
 Church, C. F., 74, 100
 Cicardo, V. H., 283, 322
 Cioranescu, M., 71 (see Hagiesco), 95
 Civin, H., 283, 322
 Clark, A. R., 60, 66
 Clark, C. L., 44, 49
 Clark, D. G., 257, 272
 Clark, I., 71 (see Porter), 98
 Clark, J. K., 43, 51
 Clark, M. C., 83 (see Harned), 95
 Clark, W. E. le G., 131, 157
 Clark, W. G., 63, 64, 66, 295, 296, 303, 304, 322
 Clarke, A. P. W., 314 (see Cleghorn), 322
 Clarkson, M. F., 86, 94
 Clausen, D. F., 63, 64, 66, 303, 322
 Cleckley, H. M., 122 (see Kruse), 142 (see Kruse), 145 (see Kruse; Sydenstricker), 159, 161
 Cleghorn, R. A., 304, 311, 314, 322, 323
 Clement, D. H., 82 (see Snyder), 99
 Cline, J. K., 72 (see Jolliffe), 96
 Coates, M. E., 88 (see Bacharach), 94, 125 (see Bacharach), 157
 Cobb, D. M., 283, 323
 Code, C. F., 294 (see Kottke), 324
 Cogswell, R. C., 127, 158
 Cohen, M. B., 87, 94
 Cohen, P. P., 265, 270
 Cohn, C., 306, 322
 Cohn, W. E., 283 (see Joseph, M.), 324
 Colbarn, R. F., 44 (see Beck), 49
 Colingsworth, D. H., 171 (see Pavcek), 220
 Collazo, J. A., 89, 94
 Collier, J., 186 (see Burkholder), 212 (see Burkholder), 215
 Collins, D. A., 90 (see Becks), 91 (see Becks), 94, 283 (see Wood), 327
 Collings, W. D., 286 (see Swingle), 327
 Colucci, D. B., 30, 49
 Compere, E. L., 280, 324
 Conklin, M. E., 238 (see Van Overbeek), 275
 Conn, J. B., 11 (see Rickes), 19 (see Rickes), 24
 Conroy, L., 42, 51
 Consolazio, F. C., 120 (see Johnson), 127 (see Egaña), 158
 Conticello, J. S., 29, 51
 Coolidge, M. H., 247 (see Somers), 274
 Cooper, D. C., 238 (see Brink), 271
 Cooper, W. C., 259, 272
 Cooper, O., 36 (see Ball), 48
 Cooperman, J. M., 62, 63, 64, 66
 Cordsen, M., 286 (see Gaunt), 323
 Corey, E. L., 279, 289, 322
 Corson, M. E., 183 (see Ryan), 191 (see Ryan), 221
 Corwin, W. C., 93 (see Ansbacher), 94
 Cosgrove, K. W., 147 (see Day, P. L.), 158
 Cosulich, D. B., 6 (see Angier; Mowat), 8 (see Mowat), 11 (see Mowat), 13

- (see Angier; Boothe; Hultquist; Waller), 14 (see Waller), 15 (see Waller), 16 (see Waller), 19 (see Angier), 22, 23, 24, 25, 85 (see Angier), 93, 198 (see Angier), 201 (see Waller), 214, 223
- Coulson, R. A., 78, 94
- Cox, W. M., Jr., 204 (see Prickett), 206, 215, 220
- Crafts, R. C., 305, 322
- Crandon, J. H., 87, 97, 117, 125, 139, 143, 158
- Craven, A., 32, 33, 34, 49
- Cravioto, B. R., 245, 246, 272
- Crawford, J. H., 128 (see Keyes), 159
- Criscola, M., 71 (see Hagiesco), 95
- Crismon, C. S., 308 (see Crismon, J. M.), 322
- Crismon, J. M., 308, 322
- Crittenden, P. J., 83, 94
- Crowder, J. A., 204 (see Anderson, R. J.), 214
- Crowe, M. O. L., 175, 215
- Csik, L., 128, 153
- Cuffaro, M., 203, 215
- Cunningham, R. W., 83 (see Harned), 95
- Cushman, M., 117, 157
- Cuthbertson, E. M., 281, 322, 323
- Cutler, E. C., 85 (see Farber), 95
- Cutler, H. H., 317, 322
- D**
- Daft, F. S., 4, 22, 29 (see Kornberg), 42 (see Kornberg), 51, 84, 85 (see Watson), 94, 100, 197, 215
- Dam, H., 28, 29 (see Tarbell), 30, 34, 35, 37, 40, 42, 43, 44, 45, 47, 49, 50, 53, 207, 208, 215, 219
- Damon, S. R., 167, 215
- Daniel, L. J., 17, 18, 22
- Dann, W. J., 106, 107, 158
- Danowski, T. S., 283, 322
- Darby, W. J., 85, 95, 106, 107, 115, 147 (see Day, P. L.), 158, 160, 212, 215
- Darling, R. C., 127 (see Egaña; Johnson), 158
- Darrow, D. C., 282, 284, 285, 288, 289, 292, 294, 298, 302, 307, 308, 309, 311, 312, 319, 322, 323, 325
- Davidson, C. S., 71, 95
- Davies, T. H., 28 (see Baker), 48
- Davis, D. M., 294, 325
- Davis, H. C., 82 (see Maroney), 97
- Davis, J. E., 86, 95
- Davis, L. J., 86, 95
- Day, H. G., 139 (see Follis), 158
- Day, P. L., 4, 17, 20 (see Laskowski), 23, 85, 95, 145, 146, 147, 158, 161, 212, 215
- Dean, R. B., 291 (see Visscher), 327
- DeEds, F., 88, 89 (see Wilson), 100
- Deeny, J., 87, 95
- Deggeller, C., Jr., 125, 158
- Delisle, A. L., 260, 274
- Delluva, A. M., 267 (see Sonne), 274
- Demole, V., 70, 76, 79, 86, 91, 95
- Denko, C. W., 127 (see Cogswell), 158
- Dennis, C., 292, 295, 322
- Dennis, J., 283 (see Mullin), 325
- Dennison, R., 259, 272
- Denny-Brown, D., 147, 158
- Derks, T. J. G., 171 (see Eijkman), 216
- DeRopp, R. S., 239, 240, 272
- Deuber, C. G., 260, 272
- Deuel, H. J., Jr., 296, 298, 299, 321, 322
- DeVaughan, N. M., 140 (see Sydenstricker), 161
- Devirian, P. S., 228, 229, 231, 270, 271
- Devyatnin, V. A., 33, 53
- Dewey, V. C., 171, 217
- DeWoody, J., 191 (see Axelrod), 214
- Dichek, M., 116, 161
- Dicken, D., 190, 193, 218
- Diehl, F., 71, 95
- Dill, D. B., 117 (see Crandon), 125 (see Crandon), 139 (see Crandon), 158
- Dittmer, K., 83 (see Kensler), 96, 190 (see du Vigneaud), 191, 215, 219, 223, 266 (see du Vigneaud), 275
- Dixon, C. E., 44 (see Clark, C. L.), 49
- Dodds, A., 47 (see Hatton), 50
- Doisy, E. A., 28, 49, 208 (see McKee; Thayer), 219, 222
- Dolan, L. A., 190 (see Duschinsky), 191 (see Duschinsky), 216
- Donnelly, M., 257, 258, 272
- Dorfman, A., 181, 216, 235, 272
- Dorland, R., 244, 248, 271
- Doxtader, E. K., 88 (see Wilson), 100

Drea, W. F., 174 (ref. 33), 178 (see Boissevain), 215
 Dreker, L., 191 (see Rubin), 220
 Drigalski, W. von, 122, 158
 Driver, R. L., 292, 322
 Droese, W., 128, 158
 Dry, T. J., 82, 95
 D'Silva, J. L., 283, 322
 Duane, R. B., 82 (see Rose), 98
 Dubnoff, J. W., 127 (see Borsook), 157
 Dufrenoy, J., 36 (see Pratt), 46 (see Pratt), 52
 Dugal, L. P., 87, 95
 Duggar, B. M., 239 (see Hildebrandt), 272
 Durand, J. I., 77, 95
 Durlacher, S. H., 312, 322
 Duschinsky, R., 190, 191, 216
 Duszynska, J., 316, 327
 Dutoit, C., 77 (see Williams, R. H.), 100
 Dyckerhoff, H., 43, 51

E

Eakin, E. A., 191, 216, 272
 Eakin, R. E., 190, 191, 216, 272
 Eastcott, E. V., 196, 197, 216
 Ebbs, J. H., 131, 158
 Ecke, R. S., 82 (see Yeomans), 100
 Eckert, J. F., 303, 326
 Edgcombe, S. W., 257, 260, 272
 Egaña, E., 77 (see Williams, R. H.), 100, 127, 158
 Eichelberger, L., 284, 324
 Eijkman, C., 171, 216
 Eisen, H. N., 80 (see Stoerk), 99
 Eisenberg, H., 314, 315 (see Thorn), 316 (see Thorn), 327
 Eisenstadt, W. S., 76, 95
 Ekstrand, T., 216
 Elion, G. B., 9, 23
 Eliot, M. M., 148, 158
 Elliott, Margaret C., 42, 49
 Ellsworth, R., 284 (see Harrop), 287 (see Harrop), 323
 Elsom, K. O., 125, 158
 Elvehjem, C. A., 20 (see Briggs), 22, 56 (see Anderson; McIntire; Schweigert), 57 (see Hegsted; McIntire), 59 (see McIntire), 60 (see Anderson; McIntire; Schweigert; Shaw), 62

(see Cooperman), 63 (see Cooperman), 64 (see Cooperman), 66, 67, 74 (see Black), 79 (see Krehl), 81 (see Black; Briggs), 94, 96, 166 (ref. 200, 211, 212), 169 (see Pavcek), 170 (see Pavcek), 171 (see Pavcek), 182 (ref. 200), 183 (see Newell), 184 (ref. 200, 327), 189 (ref. 200), 195 (ref. 225), 198 (see Mills), 199 (ref. 200), 201 (see Mills), 211 (ref. 200), 212 (see Newell), 218, 219, 220, 221, 223, 235 (see Woolley, D. S.), 275
 Emerson, G. A., 74, 95
 Emerson, K., Jr., 318 (see Thorn), 327
 Emmel, J. M., 40, 49
 Emmett, A. D., 1 (see Pfflner), 24, 84 (see Pfflner), 85 (see Pfflner), 98
 Enebo, L., 166 (ref. 201), 219
 Engel, L. L., 313, 316 (see Thorn), 327
 Ennatskaya, V. V., 203, 222
 Eppright, M. A., 169, 186, 187, 192, 194, 196, 199, 216
 Epps, H. M. R., 264, 272
 Erden, B., 9 (see Karrer), 23
 Erickson, A. E., 9 (see Weijlard), 25
 Erickson, D., 291 (see Visscher), 327
 Erickson, J., 233, 234, 271
 Eriksen, F., 39, 49
 von Euler, H., 185, 216
 von Euw, J., 314, 326
 Evans, G., 297, 300, 322
 Evans, J. S., 316 (see Ingle), 324
 Evans, V. J., 138, 139, 161
 Evans, W., 86, 95
 Eversole, W. J., 286, 314, 323
 Ewing, D. T., 33, 49
 Eylenburg, E., 42 (see Walker, S. E.), 53, 89 (see Walker), 100

F

Fahrenbach, M. J., 6 (see Angier), 13 (see Angier; Boothe; Hultquist; Waller), 14 (see Waller), 15 (see Waller), 16 (see Waller), 19 (see Angier), 22, 23, 25, 85 (see Angier), 93, 198 (see Angier), 201 (see Waller), 214, 223
 Fancher, O. E., 46, 49, 50, 93 (see Calandra; Fosdick), 94, 95

- Farber, S., 85, 95
 Farmer, C. J., 86, 87, 93, 95
 Faust, E. C., 82, 95
 Fawns, H., 169, 216
 Fazekas, J. F., 289 (see Martin), 325
 Fein, H. D., 73 (see Jolliffe), 96
 Felsher, R. Z., 261, 262, 275
 Fenn, W. O., 283, 284, 323, 325
 Fernholz, E., 30, 49
 Ferrebee, J. W., 74, 95, 314 (see Kuhlmann; Ragan), 315, 319, 323, 324, 325
 Ferregra, A. B., 35, 49
 Fetcher, E. S., Jr., 291 (see Visscher), 327
 Fielder, H., 230, 272
 Field, H., Jr., 70, 95
 Field, J. B., 34, 35, 45, 49, 50
 Fieser, L. F., 30, 31, 32, 33 (see Hershberg), 34, 50
 Filippov, V., 242, 274
 Fink, H., 166 (ref. 71), 169 (see Scheunert), 169, 170, 171, 172, 173, 184 (ref. 70), 185, 216, 221
 Firor, W. M., 318, 327
 Fischel, E. E., 82 (see Rose), 98
 Fischer, A. M., 171, 216
 Fischmann, C. F., 205 (ref. 29), 215
 Fisher, M. L., 86 (see Maurer), 97
 Fleisher, A. M., 75, 95
 Flickinger, M. H., 180 (see Wickerham), 223
 Flinn, B. C., 191 (see Axelrod), 214
 Flock, E. V., 283, 293 (see Kendall), 323, 324
 Flower, D., 190 (see Duschinsky), 191 (see Duschinsky; Rubin), 216, 220
 Foeste, A., 168 (see Parsons), 219
 Folkers, K., 5 (see Wolf), 12 (see Wolf), 25, 194 (see Harris, S. A.), 202 (see Wolf), 217, 223
 Follis, R. H., Jr., 105, 139, 158
 Foltz, E. E., 127, 157, 158
 Fontaine, M., 179, 180 (see Guilliermond), 216, 220
 Forbes, W. H., 127 (see Johnson), 158
 Fosdick, L. S., 46, 47, 49, 50, 93, 94, 95
 Fosse, R., 267, 272
 Foster, J. W., 180, 194 (see Stokes), 195 (see Stokes), 196 (see Stokes), 216, 222
 Foster, S. O., 72 (see Turner), 99
 Foster, W. C., 144, 159
 Fowells, H. A., 258, 272
 Fowler, J. L. A., 304, 314 (see Cleghorn), 322, 323
 Fradkin, R., 40 (see Smith, C. C.), 53, 93 (see Smith, C. C.), 99
 Franklin, A. L., 16, 17, 23
 Franklin, K. J., 134, 158
 Frantzell, A., 141, 158
 Franzen, R., 130, 158
 Fraps, G. S., 256, 257, 258, 272
 Frazer, L. E., 83, 98
 Fred, E. B., 205 (ref. 221, 332), 209 (see Baumann), 214, 220, 223
 Frey, C. N., 42 (see Light), 51, 89 (see Light), 97, 170 (see Schultz, A. S.), 171 (see Schultz, A. S.), 172 (see Schultz, A. S.), 183 (see Schultz, A. S.), 195 (ref. 15), 214, 221
 Freytag, R. M., 90 (see Hendricks, J. B.), 91 (see Becks), 94, 96
 Friedlander, E. W., 170, 215
 Fries, N., 180, 217
 Frisbie, H. E., 47, 52
 Fromageot, C., 209, 216
 Frostig, J. P., 71, 95
 Fry, E. G., 297 (see Long), 298 (see Long), 299 (see Long), 300 (see Long), 325
 Fry, E. M., 30 (see Fieser), 50
 Fudge, J. F., 35 (see Brown), 49, 256, 257, 258, 272
 Fujiwara, T., 80 (see Nakahara), 98
 Fukushima, D. K., 29 (see Tarbell), 53
 Fulton, M. C., 78 (see Sydenstricker), 99
- G
- Gaffron, H., 37, 38, 50
 Gairns, S., 279, 280, 289, 294, 321
 Gale, E. F., 264, 272
 Galli, A., 203, 216
 Galli, H., 203, 216
 Galston, A. W., 264, 272
 Gammelgaard, A., 44, 50
 García Merino, V., 50
 Garey, J. C., 210 (see Bauernfeind), 211 (ref. 19, 20), 214
 Garino, M., 88, 95

- Garrison, L. S., 216
 Gastpar, 134, 158
 Gatch, W. W., 40 (see Wakim), 53
 Gates, M., 9, 23
 Gaunt, R., 285, 286, 289, 290, 293, 317, 323, 324, 325
 Gautheret, R. J., 239, 272
 Gazzola, A. L., 15 (see Boothe), 19 (see Boothe), 22
 Geddes, W. F., 250, 251, 252 (see Hoffer), 253, 254, 272
 Geeslin, L., 78 (see Sydenstricker), 99
 Geiger, A., 30 (see Dam), 49
 Geiger, W. B., 36, 47, 50
 Geiger-Huber, M., 203, 216
 Geiling, E. M. K., 290, 322
 Geisinger, R., 80 (see Axelrod), 94
 Gennis, J., 72 (see Jolliffe), 96
 Genung, E. F., 168, 216
 Gerard, E., 204, 216
 Gerity, M. K., 315 (see Ferrebee), 323
 Gersh, I., 288, 323
 Gerstung, R. B., 193, 218
 Gibson, Q. H., 87 (see Barcroft), 94
 Gilberg, H., 168 (see Parsons), 219
 Gilchrist, E., 125, 160
 Gill, A. J., 284, 321
 Gillespie, J. M., 192, 220
 Gilligan, G. M., 203, 216
 Gilman, A., 285, 323
 Giral, F., 29, 50
 Gisiger, L., 257, 258, 272
 Glavind, J., 28, 29, 30 (see Dam), 37, 39, 43, 49, 50, 207 (see Dam; Orla-Jensen, S.), 208 (see Dam), 215, 219
 Göthlin, G. F., 124, 158
 Goldberg, M. S., 45, 50
 Goldstein, M. R., 85, 96
 Goldsworthy, N. E., 193 (see Lemberg), 218
 Goldzieher, J. W., 78, 95
 Gollub, M., 263, 272
 Gonzalez, Felipe, 36, 50
 Goodhart, R., 72 (see Bowman; Jolliffe), 94, 96
 Goodyear, G. H., 181 (see Williams, R. J.), 223
 Goricica, H. J., 172, 216
 Gordon, W. G., 205 (ref. 87, 88), 207, 216
 Gorham, P. R., 258, 272
 Gorlich, O., 203 (see Bernhauer), 215
 Gottlieb, B., 47, 50
 Gottlieb, D., 203, 216
 Gounelle, H., 128, 158
 Graessle, O., 42 (see Seeler), 52, 71 (see Seeler), 99
 Graeve, P. de, 267 (see Fosse), 272
 Granados, H., 42, 47, 50
 Grande, F., 122, 147, 158
 Grant, G. A., 204, 221
 Graves, B., 145, 146, 158
 Graybiel, A., 87, 95, 127 (see Egafia; Johnson), 158
 Greaves, A. V., 122 (see Adolph), 157
 Greco, D., 31, 50
 Greeley, P. O., 296 (see Bavetta), 321
 Green, D. E., 21 (see Ratner), 24, 193 (see Ratner), 198 (see Ratner), 220
 Green, M. N., 193, 221
 Greenberg, D. M., 281, 282, 283 (see Joseph, M.), 322, 323, 324
 Greene, H. C., 204 (see Wenck), 223
 Greene, J., 251, 254, 255, 256, 258, 271
 Greene, J. A., 317, 323
 Gregor, H. P., 291 (see Visscher), 327
 Gregory, F. G., 240, 272
 Greiff, D., 82, 95
 Greslin, J., 70, 79, 81, 100
 Griffith, A. S., 62, 66
 Griffith, J. Q., Jr., 89, 95
 Groat, R. A., 297, 303, 323
 Grollman, A., 288, 323
 Groody, M., 90 (see Morgan), 97
 Grossman, E. B., 72 (see Turner), 99
 Gubner, R., 46, 50
 Guest, G. M., 304, 325
 Guest, K. G. M., 41 (see Rapoport), 52
 Guest, M. M., 34 (see Ware), 53
 Guetzkow, H., 128 (see Brozek), 157
 Guha, B. C., 167, 216
 Guidarini, C., 30 (see Carrara), 49
 Guild, L. P., 245 (see Cravioto), 246 (see Cravioto), 272
 Guilliermond, A., 179, 180, 216
 Guilloud, M., 180, 221
 Guirard, B. M., 181 (see Lipmann), 194 (see Snell, E. E.), 195, 196, 218, 221, 264 (see Snell), 266 (see Lipmann), 273, 274

Gundel, M. E., 83 (see Seeler), 99
 Gunness, M., 189 (see Stokes), 191, 222
 Gunsalus, I. C., 194 (see Lichstein), 195
 (see Bellamy), 196 (see Bellamy),
 215, 218, 264, 265 (see Lichstein),
 272, 273, 275
 Gustoff, F. H., 210 (see Bauernfeind),
 211 (ref. 20), 214
 Gyntelberg, J., 44, 50
 György, P., 83, 94, 188 (see du Vigneaud),
 223

H

Haagen-Smit, A. J., 237, 240 (see Bonner,
 D. M.), 270, 273
 Haas, H. F., 216
 Haber, E. S., 257, 260, 272
 Haegge, L., (see Fenn; Noonan), 323, 325
 Haegge, L. F., 282, 325
 Haffenreffer, T. C., Jr., 166 (ref. 172), 184
 (ref. 172), 195 (ref. 172), 218
 Hagiesco, D., 71, 95
 Hague, E., 188 (see Melville), 219, 223,
 266 (see du Vigneaud), 275
 Hale, F., 194 (see Lyman), 218
 Haley, T. J., 75, 95
 Hall, W. K., 146 (see Sydenstricker), 161
 Hallman, L. F., 296 (see Bavetta), 298
 (see Deuel), 299 (see Deuel), 321, 322
 Halter, C. R., 80 (see Kensler), 96
 Hamilton, H. L., 82, 95
 Hamilton, J. W., 62, 63, 64, 66
 Hamilton, T. S., 85 (see Johnson, B. C.),
 96
 Hammond, W. H., 136 (see Adcock), 157
 Hamner, C. L., 255, 257, 258, 272
 Hamner, K. C., 247 (see Somers), 274
 Handler, P., 188, 216
 Hannah, J. R., 62 (see Rose), 66
 Hansen, C. R., 205 (ref. 214), 220
 Hansen, P. From, 45, 49, 50
 Harden, A., 187, 216
 Hardwick, S. W., 72, 94
 Hardwicke, Sarah Hooker, 43, 50
 Harington, C. R., 171, 217
 Harkevitch, N., 35, 50
 Harned, B. K., 83, 95
 Harrell, R. F., 129, 158
 Harris, J. E., 283, 323
 Harris, L. J., 74 (see Abbasy), 89, 93, 95,
 116 (see Kodicek), 159
 Harris, P. L., 91, 96
 Harris, R. S., 41 (see Lockhart; Nightin-
 gale), 51, 90, 96, 245 (see Cravioto),
 246 (see Cravioto), 272
 Harris, S. A., 5 (see Wolf), 12 (see Wolf),
 25, 194, 202 (see Wolf), 217, 223
 Harrison, D. C., 87 (see Barcroft), 94
 Harrison, H. E., 171, 217, 284, 285, 288,
 289, 292, 294, 302, 311, 319, 322, 323
 Harrison, J. H., 85 (see Farber), 95
 Harrop, G. A., 280, 281, 284, 286, 287,
 289, 302, 306, 316, 317, 323
 Hart, E. B., 20 (see Briggs), 22, 57 (see
 Hegsted), 66, 81 (see Briggs), 94
 Hartelius, V., 192, 193, 217
 Hartman, F. A., 288, 306, 312, 313, 322,
 323, 324, 326
 Hastings, A. B., 280, 281, 282, 284, 324,
 325
 Hatton, E. H., 47, 50
 Hawkins, J. W., 85 (see Farber), 95
 Hawkins, N. C., 90 (see Morgan), 97
 Hays, H. W., 286, 298 (see Parkins),
 300 (see Parkins), 320 (see Swingle),
 324, 325, 326, 327
 Heath, C., 127 (see Egaña), 158
 Hecht, A. F., 124, 158
 Hecht, E., 204, 217
 Hecht, G., 75, 96
 Hecht, S., 122, 123, 158
 Heegaard, E., 261, 272
 Hegnauer, A. H., 285, 286, 324, 326
 Hegsted, D. M., 57, 66
 Heiduschka, A., 205 (ref. 101), 206, 217
 Heinle, R. W., 85, 96
 Heller, V. G., 281, 324
 Hellman, L. M., 44, 50
 Helmholz, H. F., 304, 321
 Helve, O. E., 284, 324
 Henderson, C., 120 (see Johnson), 158
 Henderson, C. R., 127 (see Cogswell),
 158
 Hendricks, J. B., 90, 96, 98
 Hendricks, W. J., 82, 96
 Henschel, A. F., 127, 128 (see Keys), 129
 (see Taylor), 159, 161
 Heppel, L. A., 282, 303, 324
 Herrick, J. A., 167, 217

- Herrlich, H. C., 289 (see Martin), 325
 Hershberg, E. B., 33, 50
 Herzberg, B., 77, 96
 Heublein, G. W., 125 (see Elsom), 158
 Heuser, G. F., 17 (see Daniel), 18 (see Daniel), 20 (see Schumacher; Scott), 22, 24
 Hewston, E. M., 123 (see Booher), 157
 Heyl, D., 194 (see Harris, S. A.), 217
 Heyne, E. G., 257 (see Myers), 258 (see Myers), 273
 Hickey, R. J., 177, 178, 217
 Hickman, K. C. D., 91, 96
 Hicks, R. A., 71 (see Wyatt), 100
 Hightower, D. P., 80 (see Spies), 81 (see Spies), 99
 Hildebrandt, A. C., 239, 272
 Hills, G. M., 181, 217
 Himes, H. W., 195 (see Melnick), 219
 Hinton, J. J. C., 247, 272
 Hitchcock, A. E., 256, 257, 260, 272
 Hitchings, G. H., 9, 23
 Hoag, E. H., 183 (see Cheldelin), 215, 265 (see Cheldelin), 272
 Hoberg, B., 185 (see von Euler), 216
 Hoch, A., 171 (see Fink), 216
 Hochberg, M., 116 (see Melnick), 159, 168, 172, 195 (see Melnick), 217, 219
 Hock, C. W., 146 (see Sydenstricker), 161
 Hodge, H. C., 47, 50, 85, 96, 98
 Hoff, F., 87, 96
 Hoff, H. E., 308 (see Winkler), 327
 Hoffer, A., 252, 253, 254, 272
 Hoffert, D., 221
 Hoffman-Ostenhof, O., 47, 50
 Hofmann, K., 188, 190, 191, 214, 217, 219, 220, 223
 Hogan, A. G., 1, 2, 3, 23, 24, 62, 63, 64, 66, 84 (see Pffner), 85 (see Pffner), 98, 192, 197, 217, 220
 Hogarth, A. H., 134, 158
 Hoï, Buu, 39 (see Meunier), 51
 Holaday, D., 181 (see Williams, R. J.), 223
 Holly, O. M., 288 (see Sandberg), 326
 Holmes, H. N., 71, 77, 87, 96
 Holmes, R. O., 305 (see Koneff), 324
 Holt, L. E., Jr., 146 (see Albanese), 150, 157, 158
 Holten, C., 287, 324
 Honorato, C. R., 35, 50
 Horowitz, N. H., 197, 261, 272
 Houchin, O. B., 62, 63, 64, 66
 Houck, C. R., 287, 326
 Houssay, B. A., 283, 324
 Howard, B., 41, 51
 Howard, R. P., 318 (see Thorn), 327
 Howell, S. R., 60, 66
 Hoxworth, P., 87, 96
 Hubbard, L. H., 80 (see Spies), 81 (see Spies), 99
 Huber, W., 38 (see Lehmann, F. E.), 51
 Huebner, Chr. F., 41 (see Link), 51
 Huff, J. W., 263 (see Rosen), 274
 Hultquist, M. F., 6 (see Angier), 13, 14 (see Waller), 15 (see Waller), 16 (see Waller), 17 (see Seeger), 19 (see Angier), 22, 23, 24, 25, 85 (see Angier), 93, 198 (see Angier), 201 (see Waller), 214, 223
 Hunt, R., 77, 96
 Hunter, J. W., 127 (see Bransby), 157
 Hutchings, B. L., 3, 4, 6 (see Angier; Mowat), 7, 8, 9, 11 (see Mowat), 13 (see Angier; Boothe; Hultquist; Waller), 14 (see Waller), 15, 16, 19, 20, 21, 22, 23, 24, 25, 85 (see Angier; Day), 93, 95, 194 (see Bohonos), 198, 201, 214, 215, 217, 223, 235 (see Bohonos), 270
- I
- Iglesias, S., 29, 50
 Ingalls, T. H., 87, 96
 Ingle, D. J., 297, 298, 304, 311, 312, 316, 324
 Ingraham, M. A., 209, 214, 217
 Ingraham, R. C., 291, 292, 324
 Ingram, W. R., 315, 327
 Irreverre, F., 30, 50
 Isaacs, B. L., 42 (see Elliott), 49, 123, 158
 Isbell, H., 140 (see Sydenstricker), 161
 Ivy, A. C., 42 (see Elliott), 49, 113, 123 (see Isaacs), 127 (see Barborka; Foltz), 157, 158
- J
- Jackson, B., 77, 96
 Jacobs, F. A., 313 (see Olson), 325

- Jacobsen, E.**, 39 (see Eriksen), 49
Jacques, L. B., 41, 50
James, W. O., 261, 271
Jansen, K. F., 39, 40, 50
Jarnol, J., 212 (see Sullivan), 222
Javert, Carl T., 42, 50
Jeghers, H., 122 (see Wald), 161
Jensen, K. A., 39, 40, 50
Joffe, M., 91, 96
John, H. M., 80 (see Stoerk), 99
Johnson, B. C., 85, 96
Johnson, H., 89, 98
Johnson, M. J., 166 (ref. 322), 169 (see VanLanen), 170 (see VanLanen), 171 (see VanLanen), 172 (see VanLanen), 173 (see VanLanen), 223
Johnson, R. E., 117, 120, 123 (see Wald), 126, 158, 161
Johnson, R. M., 180 (see Wickerham), 223
Johnson, W. M., 79, 96
Johnston, G. W., 317 (see Greene), 323
Jolliffe, N., 72, 73, 76, 80, 94, 96, 127 (see Kruse), 131, 141 (see Adamson), 143 (see Adamson), 145 (see Adamson), 147 (see Adamson), 157, 159
Jones, C. M., 87 (see Bartlett), 94
Jones, E., 85, 95
Jones, M. J., 18, 23, 192 (see Lampen), 202 (see Lampen), 218
Jones, R. H., 135, 159
Jonxis, 149
Joseph, M., 283, 324
Joseph, S., 289, 324
Josephs, H. W., 89, 96
Jugenheimer, R. W., 257 (see Myers), 258 (see Myers), 273
Jukes, T. H., 16 (see Franklin), 20, 23, 201, 217
Jung, A., 169, 216
Jung, F. T., 123 (see Isaacs), 158
Jungeblut, C. W., 56, 60, 66
Just, F., 166 (ref. 71), 169, 170, 171, 172, 173, 184 (ref. 70), 185, 216
- K**
- Kaczka, E. A.**, 5 (see Wolf), 12 (see Wolf), 25, 202 (see Wolf), 223
Kagawa, S., 121, 159
Kahnke, J., 36 (see Armstrong, W. D.), 46 (see Armstrong, W. D.), 48, 93 (see Armstrong), 94
Kajdi, C., 121 (see Kajdi, L.), 150 (see Holt), 158, 159
Kajdi, L., 121, 159
Kalaja, L., 77, 96
Kaley, M. W., 91 (see Hickman), 96
Kallós, P., 46, 50
Kamm, O., 33 (see Ewing), 49, 192, 217
Kaplan, N. O., 181, 218, 266 (see Lipmann), 273
Kark, R., 45, 50
Karl, R. C., 78, 96
Karrer, P., 9, 23, 30, 34, 49, 50, 185 (see von Euler), 216, 217
Karrer, W., 30 (see Dam), 49
Katzin, B., 297 (see Long), 298 (see Long), 299 (see Long), 300 (see Long), 325
Kaufman, P., 91, 96
Kavanagh, F., 173, 217, 233, 234, 274
Keighley, G., 127 (see Borsook), 157
Keith, N. M., 288, 309, 310, 324
Kendall, E. C., 280, 283 (see Flock), 285, 286 (see Eversole), 293, 295, 297, 300, 305, 310, 311, 312 (see Ingle), 313, 316, 317 (see Wilder), 321, 323, 324, 327
Kensler, C. J., 80, 83, 95, 96
Kepler, E. J., 289 (see Levy), 290 (see Levy), 317 (see Levy; Robinson, F. J.; Wilder), 319 (see Tooke), 324, 326, 327
Keresztesy, J. C., 4, 5 (see Rickes), 11 (see Rickes), 12, 19 (see Rickes), 23, 24, 85 (see Lewisohn), 97, 194 (see Stiller), 198, 201, 217, 222
Keyes, P. H., 62, 66
Keys, A., 121 (see Mickelsen), 126, 127, 128, 129, 157, 159, 160, 161, 283, 324
Kidder, G. W., 171, 217
Kimmel, L., 90 (see Morgan), 97
King, A., 122, 162
King, J. D., 142, 159
King, P. S., 166 (ref. 3), 174 (ref. 3), 184 (ref. 3), 198 (see Agarwal), 199 (see Agarwal), 214
Kinosita, R., 80, 96
Kinsell, L. W., 313, 324

- Kinsey, Roy E., 44, 51
 Kiplinger, D. C., 257, 258, 273
 Klein, J. R., 188, 216
 Klinck, G. J., Jr., 87 (see McChesney), 97
 Kline, R., 298 (see Britton), 306 (see Britton), 322
 Klose, A. A., 30, 48, 207, 214
 Klotz, A. W., 183, 219
 Knight, B. C. J. G., 168, 170, 171, 173, 217, 233, 234, 273
 Knobloch, H., 181, 217
 Knopp, G., 206 (see Terroine), 222
 Knott, E. M., 74, 96
 Knutson, J. W., 36, 46, 48, 93, 94, 144, 159
 Kocher, E., 203 (see Bernhauer), 215
 Kodicek, E., 116, 159
 Kögl, F., 180, 188, 217, 237, 273
 Koenemann, R. H., 283 (see Fenn), 323
 Kofler, L., 239, 273
 Kofler, M., 30, 33, 51
 Kohler, G. O., 242, 245, 273
 Koller, F., 28, 34, 45, 50, 51
 Kolochoy, P., 175 (see Novak), 219
 Koneff, A. A., 305, 324
 Kopp, L. J., 313 (see Olson), 325
 Koppe, O., 134, 159
 Kornberg, A., 29, 42, 51
 Kornfeld, W., 130, 159
 Koser, S. A., 181 (see Dorfman), 188, 216, 217, 235 (see Dorfman), 272
 Kottke, F. J., 294, 324
 Kramer, A., 258, 273
 Krampitz, L. O., 60, 67, 173, 217
 Krebs, J., 169 (see Scheunert), 221
 Krehl, W. A., 79, 96
 Krick, E. T., 311, 326
 Kritsman, M. G., 265, 271
 Kroecker, E. H., 205 (ref. 130), 217
 Krueger, K. K., 190, 191, 217
 Kruse, H. D., 107, 108, 110, 122, 127, 141, 142, 143, 145, 146, 147, 157, 159, 161
 Kuh, E., 6 (see Angier), 13 (see Angier; Boothe; Hultquist; Waller), 14 (see Waller), 15 (see Waller), 16 (see Waller), 19 (see Angier), 22, 23, 25, 85 (see Angier), 93, 198 (see Angier), 201 (see Waller), 214, 223
 Kuhlmann, D., 314, 315, 324
 Kuhn, R., 79, 96, 183, 217
 Kuizenga, M. H., 316 (see Ingle), 324
 Kuna, S., 81, 97
 Kurland, S., 279, 321
 Kurth, E. F., 166 (ref. 132), 169, 174 (ref. 132), 182 (ref. 132), 184 (ref. 132), 189 (ref. 132), 190, 192 (ref. 132), 199, 217
 Kutz, K. L., 305, 324
- L**
- Laborey, F., 174 (ref. 147), 181, 218
 Lachaux, M., 179, 220
 Lackey, M. D., 40 (see Smith, C. C.), 63, 93 (see Smith, C. C.), 99
 Laidlaw, P., 62, 66
 Laine, T., 265, 275
 Lampen, J. O., 18, 23, 192, 193, 202, 217, 218
 Landor, J. V., 122, 159
 Landy, M., 83, 98, 189, 190, 192, 193, 218, 234, 273
 Lane, R. L., 252, 272
 Lang, R. S., 71, 100
 Lange, H. F., 119, 159
 Langston, W. C., 17 (see Day), 23
 Lanman, T. H., 87, 96
 Lapage, C. P., 87, 96
 Lardy, H. A., 218
 Larkum, N. W., 192 (see Landy), 193 (see Landy), 218
 Larsen, A., 189 (see Stokes), 200, 201, 222
 Larsen, E. G., 34 (see Field), 50
 Larsen, E. Hj., 43, 44 (see Gammelgaard), 49, 50, 51, 52
 Larson, P. S., 283, 322, 324
 LaRue, C. D., 238, 273
 Laskowski, M., 20, 23
 Laszt, L., 296, 301, 304, 324, 327
 Later, E. R., 89 (see Raiman), 98
 Lauber, H. J., 87, 96
 Laufer, L., 166 (ref. 266), 184 (ref. 266), 221
 Laufer, S., 166 (ref. 266), 184 (ref. 266), 221
 Laurie, A., 257, 258, 273
 Laves, W., 29, 30, 31, 49
 Lavollay, J., 174 (ref. 147), 181, 218

- Lawney, J. C., 122 (see Adolph), 157
 Lecoq, R., 125, 159
 Lederer, E., 209, 218
 Lee, J., 171, 219
 Lee, M. E., 168, 174 (ref. 149), 189 (ref. 149), 216
 Lee, S. B., 166 (ref. 149), 167, 168, 182 (ref. 149), 184 (ref. 149), 185, 218
 Legg, D. A., 176, 218
 Le Gier, M., 62 (see Broun), 66
 Lehmann, F. E., 38, 51
 Lehmann, J., 38, 44, 51
 Leicester, H. M., 47, 51
 Lein, J., 273
 Leitner, Z. A., 76, 97
 Leland, J., 280 (see Loeb), 281 (see Loeb), 287 (see Loeb), 289 (see Loeb), 325
 Lemberg, R., 193, 218
 Lennette, E. H., 62, 66
 Lenz, G. G., 85 (see Farber), 95
 Leong, P. C., 166 (ref. 1), 168, 214
 Leonian, L. H., 169, 191, 218
 Lepp, Erica, 41, 50
 Leslie, J. D., 211 (ref. 2), 212, 214
 Lester, D., 41, 51
 Leuchtenberger, C., 85 (see Lewisohn), 97
 Leuchtenberger, R., 85 (see Lewisohn), 97
 Levine, H., 172, 216, 317 (see Greene), 323
 Levine, M., 273
 Levine, M. N., 250, 251, 272
 Levine, R., 306 (see Cohn, C.), 322
 Levitas, N., 116 (see Perlzweig), 160
 Leviton, A., 177, 178, 218
 Levy, M. D., 82, 97
 Levy, M. S., 289, 290, 317, 324
 Levy, S., 42, 44, 51
 Lewis, J. C., 166 (ref. 156, 157), 169, 173, 174 (ref. 157), 179, 182 (ref. 157), 186, 189 (ref. 157), 190, 192, 193, 194, 195, 196, 197, 199, 218, 222
 Lewis, L. A., 288 (see Hartman; Smith, D. E.), 313 (see Hartman), 323, 324, 326
 Lewis, R. A., 314 (see Thorn), 315 (see Thorn), 316 (see Thorn), 327
 Lewis, R. W., 202, 218
 Lewis, T., 128, 142, 159
 Lewis-Faning, E., 127, 160
 Lewisohn, R., 85, 97
 Lewy, F. H., 125 (see Elsom), 158
 Libowitzky, O., 87, 97
 Lichstein, H. C., 189, 194, 218, 265, 267, 273
 Lih, J., 203, 218
 Liebmann, J., 125, 159
 Light, A. E., 281, 324
 Light, J., 121 (see Kajdi, L.), 159
 Light, R. F., 42, 51, 89, 97
 Lih, B., 80, 98
 Lilienthal, J. L., Jr., 87 (see Graybiel), 95
 Liling, M., 286 (see Gaunt), 323
 Lillie, R. J., 199, 218
 Lilly, V. G., 169, 191, 218
 Lindauer, M. A., 89, 95
 Lindner, H., 205 (ref. 101), 206, 217
 Link, K. P., 34, 38, 41, 49, 50, 51, 81 (see Black), 94
 Lipmann, F., 181, 183, 218, 219, 262, 266, 273
 Livingston, P. C., 124, 159
 Livingstone, S. M., 86, 97
 Livshits, M. I., 169, 218
 Lloyd, B. B., 157
 Lockhart, E. E., 41, 51, 245 (see Cravioto), 246 (see Cravioto), 262, 272, 273
 Lockhart, H. S., 245 (see Cravioto), 246 (see Cravioto), 272
 Loeb, R. F., 279, 280, 281, 287, 289, 306, 311, 314 (see Kuhlmann; Ragan), 315 (see Ferrebee; Kuhlmann), 316, 319 (see Ferrebee), 323, 324, 325
 Lohman, K., 261, 273
 Loman, J., 70, 78, 97
 Long, B., 266 (see du Vigneaud), 275
 Long, C. N. H., 297, 298, 299, 300, 325
 Long, E. B., 223
 Loo, S. W., 238, 241, 248, 273
 Loomis, E. C., 34 (see Seegers), 52
 Lopes Cardozo, E., 319, 321
 Lopez, J. A., 73 (see Lowry), 97, 121 (see Lowry), 159
 Lord, J. W., Jr., 45, 51, 72, 97
 Lowenstein, B. E., 35 (see Oppenheimer), 52, 313, 325, 327
 Lowry, O. H., 73, 97, 117 (see Bessey), 121, 141 (see Adamson), 143 (see Adamson), 145 (see Adamson), 147 (see Adamson), 157, 159, 313 (see Talbot), 327

Lucas, G. H. W., 279, 325
 Lucia, S. P., 71, 97
 Luckey, T. D., 20 (see Briggs), 22, 81
 (see Briggs), 94, 198 (see Mills), 201
 (see Mills), 219
 Lüscher, M., 38 (see Lehmann, F. E.), 51
 Lund, C. C., 87, 97, 117 (see Crandon),
 125 (see Crandon), 139 (see Cran-
 don), 158
 Lunde, G., 203, 218
 Lundegårdh, H., 257, 258, 273
 Lundin, H., 166 (ref. 201), 219
 Lutz, A., 167, 168, 218
 Lwoff, A., 263, 273
 Lwoff, M., 263, 273
 Lyman, C. M., 181 (see Williams, R. J.),
 194, 218, 223
 Lyons, R. N., 33, 51

M

Ma, R., 191, 220
 McCance, R. A., 115, 159, 161
 McCarter, J. R., 37, 53, 208, 223
 McCay, C. M., 60 (see Howell), 66
 McChesney, E. W., 87, 97
 McClary, J. E., 230, 273
 McClendon, J. F., 104, 144, 159
 McClung, L. S., 176, 177, 221
 McCollum, E. V., 139 (see Follis), 158,
 281, 325
 McCormick, W. J., 128, 159
 MacCorquodale, D. W., 208 (see McKee;
 Thayer), 219, 222
 McCoy, R. H., 80 (see Axelrod), 94
 McCrea, F. D., 78, 97
 McCreary, J. F., 79 (see Tisdall), 99, 133
 (see Tisdall), 161
 MacDonald, H., 71, 95
 MacDonough, J. V., 166 (ref. 172), 184
 (ref. 172), 195 (ref. 172), 218
 McElroy, L., 28 (see Baker), 48
 McEwen, H. D., 311, 321
 Machella, T. E., 144, 159
 McHenry, E. W., 115, 160, 162
 McIlwain, A. J., 71 (see Maxfield), 80
 (see Maxfield), 97
 McIlwain, H., 36, 51
 McIntire, F. C., 166 (ref. 180), 167, 174
 (ref. 180), 182 (ref. 180), 190, 219
 McIntire, J. M., 56, 57, 59, 60, 66, 67
 Mack, P. B., 148, 159
 Mack, R., 32 (see Rosin), 52
 MacKay, E. M., 295, 296, 301, 312, 322,
 325
 MacKay, L. L., 312 (see MacKay,
 E. M.), 325
 McKee, R. W., 208, 219, 222
 McKelvey, J. L., 85 (see Watson), 100
 Mackenzie, A., 134, 159
 McKeown, R., 305 (see Kutz), 324
 McKibben, J. M., 74 (see Black), 94,
 184 (ref. 327), 223
 McLester, J. S., 127 (see Kruse), 159
 McMurray, J., 87 (see Barcroft), 94
 Macri, C., 42, 50
 McVeigh, I., 167, 168, 186, 200 (see
 Burkholder), 201 (see Burkholder),
 215, 242, 244, 247, 248, 252, 253,
 271, 273
 Madden, R. J., 235 (see Woolley, D. S.),
 275
 Mage, H. E., 127 (see Bransby), 136,
 157, 159
 Maguigan, W. A., 204, 206, 207, 219
 Maier, J., 82 (see Snyder), 99
 Maisel, F. E., 79, 97
 Maizel, B., 169, 219
 Mallette, M. F., 9, 17, 22, 23
 Mandelbaum, J., 122, 158
 Manery, J. F., 281, 282, 283 (see Fenn),
 323, 325
 Mangun, G. H., 282 (see Muntwyler),
 284 (see Muntwyler), 285 (see
 Muntwyler), 325
 Mann, F. C., 283 (see Flock), 293 (see
 Kendall), 323, 324
 Manning, P. D. V., 20, 24
 Marcussen, P. V., 44 (see Gammelgaard),
 50
 Marenzi, A. D., 283, 324
 Marine, D., 279, 281, 325
 Marmoy, C. J., 274
 Maroney, J. W., 82, 97
 Marrack, J. R., 74 (see Abbaay), 93
 Marsh, M., 56, 66
 Marshall, E. K., Jr., 7, 10, 21, 22, 294,
 325
 Martin, G. J., 16, 17, 23
 Martin, S. J., 289, 325

- Martinson, E. E., 31, 51
 Marx, R., 43, 51
 Mason, H. L., 72 (see Williams, R. D.),
 74, 97, 100, 147 (see Williams, R. D.),
 162, 305 (see Kendall), 310 (see
 Kendall), 324
 Massengale, O. N., 204 (see Prickett),
 205 (ref. 26), 206, 215, 219, 220
 Massock, H. E., 166 (ref. 176, 177), 178,
 181, 184 (ref. 176, 177), 186, 187,
 189 (ref. 176), 190, 219, 222
 Mathieson, D. R., 286, 324
 Mattill, H. A., 64, 66, 91, 98
 Maurer, S., 86, 97
 Mautz, F. R., 282 (see Muntwyler), 284
 (see Mellors; Muntwyler), 285 (see
 Muntwyler), 325
 Maxfield, J. R., Jr., 71, 80, 97
 Mayer, R. L., 174 (ref. 179), 178, 180, 219
 Mazoué, H., 125 (see Lecoq), 159
 Mead, M. W., Jr., 171, 219
 Meade, R. E., 174 (ref. 183), 177, 210,
 219, 220
 Mecchi, E., 207 (see Almquist), 208
 (see Almquist), 214
 Meerovich, G. I., 31, 32, 34, 51
 Meiklejohn, A. P., 127 (see Egafña), 158
 Melass, V. H., 86, 97
 Meller, C. L., 80, 97
 Mellors, R. C., 282 (see Muntwyler), 284,
 285 (see Muntwyler), 325
 Melnick, D., 116, 159, 168 (see Hoch-
 berg), 172 (see Hochberg), 195, 217,
 219, 221
 Melton, G., 79, 97
 Melville, D. B., 188, 190 (see du Vig-
 neaud), 191, 215, 219, 223
 Menotti, A. R., 29, 30, 31, 51
 Mentzer, C., 29, 39 (see Meunier), 40, 51
 Métivier, V. M., 122, 160
 Meunier, P., 29, 39, 40, 51
 Meyer, B. J., 56, 66
 Meyer, D. B., 56, 66
 Meyer, E., 147, 160
 Meyer, H. H., 69, 70, 97
 Meyer, J. R., 256, 259, 273
 Meyer, L. M., 85, 97
 Meyer, M. B., 147, 160
 Meyer, O. O., 41, 51
 Meyer, R. K., 56, 66
 Mezera, R. A., 62 (see Brown), 66
 Mickelsen, O., 117, 121, 127 (see Keys),
 128 (see Brozek; Keys), 129 (see
 Taylor), 157, 159, 160, 161, 184
 (ref. 327), 223
 Middleton, T. R., 88 (see Bacharach), 94,
 125 (see Bacharach), 157
 Mikhlin, D. M., 29, 51
 Milam, D. F., 115, 160
 Milgram, E. Y., 46, 50
 Miller, A. K., 200, 202, 219
 Miller, D., 229, 273
 Miller, E. S., 296 (see Barnes), 301 (see
 Barnes), 321
 Miller, H. C., 282, 302, 308, 309, 325
 Miller, O. N., 120, 160
 Miller, S. H., 125 (see Rapaport), 160
 Milligan, E. H. M., 127, 157, 160
 Mills, C. A., 74, 76, 97
 Mills, R. C., 57 (see Hegsted), 66, 81 (see
 Briggs), 94, 198, 201, 219
 Mims, V., 4 (see Day), 20 (see Laskow-
 ski), 21, 23, 85 (see Day), 95, 265
 (see Schales, O.), 274
 Minarik, C. E., 258, 273
 Miner, C. S., 175, 219
 Minnum, E. C., 257, 258, 259, 273
 Miranda, F. de P., 245 (see Cravioto),
 246 (see Cravioto), 272
 Mirimanoff, A., 179, 219
 Mitchell, H. H., 85 (see Johnson, B. C.),
 96, 116, 130, 160
 Mitchell, H. K., 186, 215, 219, 223, 263
 (see Beadle), 270
 Mitchell, J. S., 38, 51
 Mitchell, W. R., 218
 Mitra, K., 144, 160
 Mitrani, M. M., 76, 97
 Moe, G. K., 283 (see Wood), 313, 327
 Moggridge, R. C. G., 171, 217
 Moglia, J. L., 283, 322
 Molitor, H., 69, 70, 73, 75, 76, 81, 92,
 97
 Moller, E. F., 183, 223
 Molotchick, M. B., 92, 97
 Moore, B., 28, 51
 Moore, C. V., 85, 97
 Moore, D. F., 122, 160
 Moore, H. N., 180, 219
 Moore, M., 30 (see Fernholz), 49

- Moore, P. E., 141 (see Adamson), 143
 (see Adamson), 145 (see Adamson),
 147 (see Adamson), 157
- Moore, T., 42, 51, 52, 53, 89, 95, 98, 100
- Moosnick, F. B., 86, 97
- Moragues, V., 82 (see Greiff), 95
- Morawitz, P., 87, 97
- Morel, G., 239, 273
- Morel, M., 188, 219
- Morell, T., 128, 160
- Morgan, A. F., 90, 96, 97, 98
- Morgenstern, M., 76, 99
- Mori, K., 80 (see Nakahara), 98
- Morista, S., 224
- Morris, H. D., 257, 258, 275
- Mortarotti, T. G., 88 (see Wilson), 89
 (see Wilson), 100
- Moseley, O., 194 (see Lyman), 218
- Moss, J., 16, 17, 23
- Moss, W. G., 35, 51
- Most, R. M., 131, 159
- Mowat, J. H., 6, 7, 8, 9, 11, 13 (see
 Angier; Boothe; Hultquist; Waller),
 14 (see Waller), 15, 16, 19, 21 (see
 Hutchings), 22, 23, 24, 25, 85 (see
 Angier), 93, 198 (see Angier; Hutch-
 ings), 201 (see Waller), 214, 217, 223
- Moyer, D., 186 (see Burkholder), 212
 (see Burkholder), 215
- Moyle, W. J., 131 (see Ebbs), 158
- Mozingo, R., 5 (see Wolf), 12 (see Wolf),
 25, 202 (see Wolf), 223
- Mueller, J. H., 183, 219
- Muether, R. O., 62 (see Broun), 66
- Mull, R. P., 166 (ref. 326), 170 (see
 Vinson), 223
- Mullin, F. J., 283, 325
- Mullins, L. J., 283 (see Fenn), 323
- Muntwyler, E., 282, 284, 285, 325
- Murayama, M., 282 (see Greenberg),
 323
- Murdock, E. T., 87 (see Deeny), 95
- Murlin, J. R., 127, 128, 157
- Murneek, A. E., 256, 273
- Murphy, T. D., 43, 51
- Murray, E. S., 82, 98, 99, 100
- Murray, S., 298 (see Deuel), 299 (see
 Deuel), 322
- Mushett, C. W., 42, 51, 52, 71 (see
 Seeler), 93, 98, 99
- Myers, C. S., 305 (see Kendall), 310 (see
 Kendall), 324
- Myers, H. E., 257, 258, 273
- Myerson, A., 70 (see Loman), 78 (see
 Loman), 97
- Myrbäck, K., 172, 219
- N
- Nafziger, H., 87 (see Lauber), 96
- Najjar, V. A., 165, 184 (ref. 199), 219
- Nakahara, W., 80, 98
- Necheles, H., 89 (see Raiman), 98
- Neipp, L., 256, 273
- Neuman, M. W., 85, 98
- Neuwahl, F. J., 79, 98
- New, J. C., 78 (see Sydenstricker), 99
- Newbold, H. L., 88, 98
- Newell, G. W., 166 (ref. 200), 182 (ref.
 200), 183, 184 (ref. 200), 189 (ref.
 200), 199 (ref. 200), 211 (ref. 200),
 212, 219
- Newman, M. S., 207, 214
- Nicholls, J., 139, 161
- Nicholls, L., 122, 160
- Nicholson, W. M., 280 (see Harrop), 281
 (see Harrop), 323
- Nielsen, N., 208 (see Dam), 215
- Nightingale, G., 41, 51
- Nilson, H. W., 280 (see Allers), 302, 310,
 312 (see Ingle), 321, 324, 325
- Nilsson, R., 166 (ref. 201), 219
- Nobécourt, P., 239, 273
- Nobel, A., 134
- Noble, W. M., 166 (ref. 157), 169 (see
 Lewis, J. C.), 174 (ref. 157), 179 (see
 Lewis, J. C.), 182 (ref. 157), 189
 (ref. 157), 190 (see Lewis, J. C.),
 193 (see Lewis, J. C.), 194 (see
 Lewis, J. C.), 195 (ref. 157), 197
 (see Lewis, J. C.), 218, 222
- Noggle, G. R., 259, 273
- Noonan, T. R., 283, 323, 325
- Nord, F. F., 166 (ref. 326), 170, 223
- Norris, L. C., 17 (see Daniel), 18 (see
 Daniel), 20 (see Schumacher; Scott),
 22, 24
- Northey, E. H., 6 (see Angier), 13 (see
 Angier; Boothe; Hultquist; Waller),
 14 (see Waller), 15 (see Waller), 16

(see Waller), 19 (see Angier), 22,
23, 25, 85 (see Angier), 93, 198 (see
Angier), 201 (see Waller), 214, 223
Novak, A. F., 175, 219
Novelli, A., 29, 33, 51
Novelli, G. D., 181, 183, 218, 219, 266, 273
Nuckolls, J., 47, 52
Nungester, W. J., 62, 67
Nutter, M. K., 245 (see Cravioto), 246
(see Cravioto), 272
Nyc, J. F., 186, 215, 219, 263 (see
Beadle), 270

O

Oakwood, T. S., 204 (see Zook), 205
(ref. 360), 224
Oberle, E. A., 297, 298, 316 (see Ingle),
324
Ochoa, S., 262, 273
O'Dell, B. L., 1 (see Pfiffner), 2 (see
Pfiffner), 3 (see Pfiffner), 5 (see
Wittle), 6, 7, 19 (see Pfiffner), 20
see (Pfiffner), 24, 25, 84 (see Pfiff-
ner), 85 (see Pfiffner), 98, 198 (see
Pfiffner), 201 (see Pfiffner), 220
Odintsova, E. N., 166 (ref. 205), 169, 171,
219
Oeder, G., 130, 160
O'Kane, D., 167, 185, 219
Okunuki, K., 262, 265, 273
Olcott, H. S., 91, 98
Oleson, J. J., 4, 16 (see Hutchings), 19,
20, 23, 24
Olson, R. E., 313, 325
Ondratschek, K., 255, 273, 274
Onslow, M. W., 267, 274
Oppenheim, A., 80, 98
Oppenheimer, B. S., 35, 52
Orent-Keiles, E., 281, 325
Orla-Jensen, A. D., 207 (see Dam; Orla-
Jensen, S.), 208 (see Dam), 215,
219
Orla-Jensen, S., 207, 208 (see Dam), 215,
219
Orr, J. B., 114, 160
Oser, B. L., 87, 99, 116 (see Melnick), 159,
168 (see Hochberg), 172 (see Hoch-
berg), 195 (see ref. 270; Melnick),
217, 219, 221
Osterberg, A. E., 309 (see Keith), 324
Oswald, E. J., 192 (see Landy), 193 (see
Landy), 218
Ott, W. H., 83 (see Seeler), 99
Overman, R. S., 41 (see Link), 51, 81
(see Black), 94
Owen, C. A., 43 (see Sells), 52
Owen, P. S., 74 (see Ferrebee), 95
Owren, P. A., 34, 52
Oxford, A. E., 204, 205 (ref. 209), 219

P

Page, R. C., 70, 98
Palladin, A., 45, 52
Pallister, R. A., 122, 159, 160
Palmer, J., 313 (see Kinsell), 324
Palmer, L. S., 245, 275
Park, E. A., 148, 158
Parker, D., 74 (see Ferrebee), 95, 315
(see Ferrebee), 323
Parker, E. R., 255, 257, 274
Parkins, W. M., 280 (see Swingle), 286,
289 (see Swingle), 294 (see Swingle),
298, 300, 320 (see Swingle), 325, 326,
327
Parks, J., 44, 52
Parrott, E. M., 2, 23
Parson, W., 316 (see Loeb), 325
Parsons, H. T., 168, 219
Patterson, J. M., 115, 160
Patton, E. W., 74 (see Youmans), 100,
117, 162
Paul, H., 281, 324
Pavcek, P. L., 166 (ref. 211, 212), 169,
170, 171, 220
Pavlinova, O. A., 184 (ref. 223), 220
Paye, R. S., 44, 49
Peabody, G. E., 78 (see Karl), 96
Pearce, H., 79 (see Tisdall), 99, 133 (see
Tisdall), 161
Pearse, H. L., 260, 274
Pearson, P. B., 59, 67, 86 (see Melass),
97
Peck, R. L., 205 (ref. 214), 220
Peirce, E. C., 2nd, 85 (see Farber), 95
Peltier, G. L., 181, 220
Pentler, C. F., 207 (see Almquist), 208
(see Almquist), 214
Peraita, M., 122, 147, 158

- Perla, D., 75, 76, 98, 288 (see Sandberg), 326
- Perlzweig, W. A., 116, 121 (see Cayer), 144 (see Cayer), 157, 160, 263 (see Rosen), 274
- Petering, H. G., 9, 24
- Peters, F., 203, 215
- Peters, H. C., 292, 325
- Peters, J. P., 284, 325
- Peters, M., 82, 96
- Petersen, W. E., 86 (see Moosnick), 97
- Peterson, M. S., 173, 220
- Peterson, W. H., 166 (ref. 3, 180, 200, 211, 212, 272, 322), 167 (see McIntire), 168 (see Tatum), 169 (see Pavcek; VanLanen), 170 (see Pavcek; VanLanen), 171 (see VanLanen), 172 (see VanLanen), 173, 174 (ref. 3, 18, 180, 272, 319, see Pavcek), 175, 178 (see Singh), 182 (ref. 180, 200), 183 (see Newell; Snell, E. E.; Underkoffler), 184 (ref. 3, 200, 272), 187 (see Singh), 189 (ref. 200, 272), 190, 191, 192 (see Lampen), 193 (see Lampen), 194 (see Bohonos), 198 (see Agarwal), 199 (see Agarwal; Singh; ref. 200), 200, 204 (see Wenck), 205 (ref. 130, 221, 332), 211 (ref. 200), 212 (see Newell), 214, 215, 217, 219, 220, 221, 222, 235 (see Bohonos), 265 (see Underkoffler), 270, 275
- Pett, L. B., 115, 123, 160, 178, 220
- Pfannenstiel, W., 90, 98
- Pfeiffer, S. E., 174 (ref. 301), 179 (see Tanner), 182 (see Tanner), 183 (see Tanner), 184 (ref. 301), 189 (ref. 301), 195 (ref. 301), 210 (see Tanner), 211 (ref. 301), 212 (see Tanner), 222
- Pfiffner, J. J., 1, 2, 3, 5 (see Wittle), 6 (see O'Dell), 7, 19, 20, 22, 24, 25, 84, 85, 98, 197, 198, 201, 220, 280 (see Swingle), 289 (see Swingle), 294 (see Swingle), 320 (see Swingle), 326, 327
- Phillips, P. H., 56 (see Anderson; Schweigert), 60 (see Anderson; Schweigert; Shaw), 66, 67
- Phyfe, P., 314 (see Ragan), 325
- Pick, E. P., 76, 77, 98, 100
- Pickering, G. W., 128 (see Lewis), 159
- Pickering, V. L., 36 (see Pratt), 46 (see Pratt), 52
- Piersma, H. D., 180, 220
- Pilgrim, F. J., 191, 220, 223
- Pillat, A., 146, 160
- Pincus, P., 47, 52
- Pinder, J. L., 30, 32, 52
- Pines, K. L., 340 (see Zwemer), 327
- Pinkerton, H., 82 (see Greiff), 95
- von Pirquet, 134
- Platt, B. S., 141 (see Adamson), 143 (see Adamson), 145 (see Adamson), 147 (see Adamson), 157
- Plotz, H., 82 (see Hamilton, H. L.), 95
- Plum, C. M., 39 (see Eriksen), 49
- Plum, P., 43, 44, 49, 50, 52, 53
- Pollack, M. A., 80, 98
- Pollard, H. L., 174 (ref. 183), 177, 210 (see Meade), 219, 220
- Pollard, N., 257, 258, 259, 274
- Ponder, E., 285, 325
- Popkin, G. L., 78, 95
- Popp, W. C., 80, 100
- Porter, C. C., 71, 98
- Portis, S., 128 (see Rosenbaum), 160
- Poser, E. F., 78, 98
- Positano, G., 70, 98
- Potter, R. L., 218
- Potts, E., 60, 67
- Poulsen, J. E., 44, 52
- Power, M. H., 147 (see Williams, R. D.), 162, 289 (see Levy), 290 (see Levy), 317 (see Cutler; Levy; Robinson, F. J.; Willson), 319 (see Tooke), 322, 324, 326, 327
- Prados, M., 108, 160
- Pratt, E. F., 223
- Pratt, R., 36, 46, 52
- Prelog, V., 204, 223
- Prescott, F., 76, 94
- Preuss, L. M., 205 (ref. 221), 220
- Prickett, P. S., 204, 205 (ref. 26), 206, 215, 219, 220
- Proskuryakov, N. I., 184 (ref. 223), 220
- Pulkki, L. H., 247, 274
- Pulver, R., 233, 325
- Purmann, R., 8, 24
- Puutula, K., 247, 274

Q

- Quetelet, 130
Quick, A. J., 29, 34, 40, 41, 42, 52

R

- Rabinowitz, J. C., 195, 220
Raffy, A., 179, 180 (see Guilliermond), 216, 219, 220
Ragan, C., 314, 315 (see Kuhlmann), 319 (see Ferree), 323, 324, 325
Raiman, R. J., 89, 98
Raistrick, H., 204, 205 (ref. 209), 219
Ralli, E. P., 305, 326
Ramaswamy, S., 206, 220
Randall, R. M., 146 (see Albanese), 157
Rapaport, H. G., 125, 160
Rapoport, S., 41, 52, 304, 325
Ratner, S., 21, 24, 193, 198, 220
Rautanen, N., 265, 274
Ravel, J. M., 183, 220
Ravenel, S. F., 82, 98
Ray, S. N., 74 (see Abbasy), 93
Reames, H. R., 235 (see Dorfman), 272
Redish, M. N., 41 (see Shapiro), 53
Reed, C. I., 90, 91, 98
Reese, J. D., 305 (see Koneff), 324
Reeves, A. M., 257, 274
Reeves, R. J., 80, 98
Rehberg, P. B., 287, 324
Reichelt, E., 167, 220
Reichstein, T., 314, 326
Remington, J. W., 286, 294, 314, 323, 326, 327
Renaud, J., 179, 220
Renshaw, R. R., 77, 96
Renvall, S., 172, 221
Rettger, L. F., 175, 223
Reuss, J. S., 38, 51
Rhoads, C. P., 80 (see Kensler), 83, 95, 96, 98
Rhyn, E., 118, 157
Rice, K. K., 315, 326
Rich, W. M., 122, 160
Richards, R. K., 82, 98
Richardson, E. M., 232, 271
Richardson, L. R., 35 (see Brown), 49
Richert, D., 313 (see Olson), 325
Richert, Dan A., 34, 52
Richter, C. P., 303, 315, 326
Rickes, E. L., 4 (see Keresztesy), 5, 11, 19, 23, 24, 198 (see Keresztesy), 201 (see Keresztesy), 217
Riegel, Byron, 28, 52
Rigdon, R. H., 40, 52
Riker, A. J., 166 (ref. 180), 167 (see McIntire), 174 (ref. 180), 182 (ref. 180), 190 (see McIntire), 219, 239 (see Hildebrandt), 272
Riley, R. L., 87 (see Graybiel), 95
Rinehart, J. F., 89, 98
Rinkel, M., 70 (see Loman), 78 (see Loman), 97
Robbins, E. B., 81, 99
Robbins, M., 20 (see Bird), 22
Robbins, W. J., 180, 191, 220, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 274
Roberts, L. J., 123, 157
Robertson, J. E., 71 (see Maxfield), 80 (see Maxfield), 97
Robinson, A., 281 (see Orent-Keiles), 325
Robinson, E. J., 285, 286, 324, 326
Robinson, F. J., 317, 327
Robinson, H. J., 92, 97
Robinson, H. L., 122 (see Adolph), 157
Robinson, J., 116 (see Perlzweig), 160
Robinson, P., 77 (see Williams, R. H.), 100
Robinson, P. F., 120 (see Johnson), 158
Robinson, W. D., 70, 95
Roch, M., 72, 98
Rodahl, K., 42, 52, 89, 98
Rodbart, R., 174 (ref. 179), 178, 219
Roderick, L. M., 38, 52
Rodgers, N. E., 174 (ref. 183, 236), 177, 210 (see Meade), 219, 220
Rodgers, T. S., 127 (see Branshy), 157
Rodriguez, J. S., 89, 94
Röhrer, 130
Roepke, R. R., 192 (see Lampen), 202 (see Lampen), 218
Rogan, J. J., 87 (see Deeny), 95
Rogers, L. L., 189, 216, 220, 221
Rogoff, J. M., 279, 280, 326
Rohner, F., 178, 220
Rohrmann, E., 223, 265, 275
Roscoe, M. H., 203, 216

- Rose, C. L., 62, 66
 Rose, H. M., 82, 98
 Rosen, F., 116 (see Perlzweig), 160, 191
 (see Rubin), 220, 263, 274
 Rosenbaum, E. E., 128, 160
 Rosenberg, G., 89, 100
 Rosenberg, H. R., 28, 52
 Rosenblum, H., 32 (see Rosin), 52, 83,
 98
 Rosenblum, L. A., 73 (see Jolliffe), 80
 (see Jolliffe), 96
 Rosin, J., 32, 52
 Ross, B. D., 90 (see Harris, R. S.), 96
 Rothman, S., 87, 98
 Rothschild, E., 30 (see Dam), 49
 Rothschild, P., 128 (see Lewis), 159
 Roubaix, J. de, 257, 271
 Roulet, F., 178, 220
 Routh, J. I., 62, 63, 66
 Rowntree, L. G., 286, 326
 Rózsa, P., 31, 32, 52
 Rubbo, S. D., 192, 220
 Rubin, M. I., 311, 326
 Rubin, S. H., 190 (see Duschinsky), 191,
 216, 220
 Ruckstuhl, H., 185 (see von Euler), 216
 Rudert, F. J., 180, 220, 222
 Rudesill, C. L., 80, 98
 Ruffin, J. M., 121 (see Cayer), 127, 144
 (see Cayer), 157, 160
 Ruggieri, G., 70, 98
 Ruskin, S. L., 88, 98
 Rusznyak, St., 88, 98
 Rutschman, J., 217
 Ryan, A. E., 87 (see Bartlett), 94
 Ryan, F. J., 183, 191, 221
 Ryle, J. A., 113, 134, 160
 Rynearson, E. H., 317 (see Wilder), 327
 Ryon, J. B., 87, 99
 Rytz, W. von, Jr., 242, 245, 246, 251,
 274
- S
- Sah, P. P. T., 36 (see Pratt), 46 (see
 Pratt), 52
 Sakurai, K., 169, 221
 Salomon, H., 30 (see Dam), 49, 185 (see
 von Euler), 216
 Sampson, W. L., 29, 53, 73, 75, 97
 Samuels, L. T., 298 (see Deuel), 299 (see
 Deuel), 322
 Sandberg, M., 76, 98, 288, 326
 Sanders, E. K., 44, 53
 Sandground, J. H., 82, 98
 Sandstead, H. R., 144, 160
 Sarett, H. P., 183 (see Cheldelin), 201,
 215, 221, 265 (see Cheldelin), 272
 Sarma, P. S., 195 (ref. 225), 221
 Saudck, E. E., 171 (see Pavcek), 220
 Saunders, A., 176, 177, 221
 Saunders, F., 235 (see Dorfman), 272
 Savard, K., 204, 221
 Sawhill, J., 80 (see Jolliffe), 96
 Scarborough, H., 125, 160
 Schade, H. A., 86, 98
 Schaffstein, G., 259, 274
 Schales, O., 265, 274
 Schales, S. S., 265 (see Schales, O.), 274
 Schamp, H. M., 309, 326
 Scheel, L. D., 41 (see Link), 51
 Scheiffly, C. H., 82 (see Dry), 95
 Scheunert, A., 166 (ref. 248, 251), 167,
 168, 169, 171, 221
 Schiebllich, M., 166 (ref. 251), 167, 168,
 169, 171, 221
 Schittenhelm, A. Z., 267, 274
 Schivek, A. I., 190, 221
 Schlack, C. A., 60 (see Howell), 66
 Schleicher, E. M., 86 (see Moosnick), 97
 Schlenk, F., 194, 221
 Schmidt, F. R., 79, 98
 Schmidt, H. L., 78 (see Sydenstricker), 99
 Schmidt, J. L., 83, 98
 Schmidt, M. B., 180, 220, 228, 229, 274
 Schmidt, Th., 37, 52
 Schneider, L. K., 183 (see Ryan), 191
 (see Ryan), 221
 Schoeffel, E. W., 86 (see Maurer), 97
 Schoen, K., 33, 52
 Schoenheimer, R., 204 (see Anderson,
 R. J.), 214
 Schonberg, K., 173, 221
 Schopfer, W. H., 170, 171, 174 (ref. 256),
 180, 209, 221, 226, 233, 274
 Schrader, A. L., 258, 273
 Schroeder, A. R., 283 (see Brewer), 322
 Schüller, H., 130, 159
 Schukina, L. A., 34 (see Shemiakin), 53
 Schulek, E., 31, 32, 52

- Schultz, A. S., 170, 171, 172, 183, 195
(ref. 15), 214, 221
- Schultz, E. M., 191, 221
- Schultz, H. W., 174 (ref. 33), 178 (see Boissevain), 215
- Schumacher, A. E., 20, 24
- Schuster, P., 261, 273
- Schwartz, B. M., 287, 326
- Schwarz, H., 35, 52, 93, 99
- Schwarz, R., 166 (ref. 266), 184 (ref. 266), 221
- Schweigert, B. S., 56, 57, 59, 60, 66, 67
- Schweizer, M., 286 (see Gaunt), 289
(see Joseph, S.), 323, 324
- Schwyzer, R., 9 (see Karrer), 23
- Sciclounoff, F., 72, 98
- Scott, C. C., 81, 99
- Scott, E. G., 82 (see Maroney), 97
- Scott, H. H., 122, 160
- Scott, M. L., 17 (see Daniel), 18 (see Daniel), 20, 22, 24
- Scott, W. A., 131 (see Ebbs), 153
- Seudi, J. V., 31, 32, 34, 36, 52
- Sebrell, W. H., 4, 22, 29 (see Kornberg), 42
(see Kornberg), 51, 70, 72, 78, 84, 85
(see Watson), 94, 99, 100, 122 (see Kruse), 141 (see Adamson), 142
(see Kruse), 143 (see Adamson), 144,
145 (see Adamson; Kruse; Sydenstricker), 147 (see Adamson), 157,
159, 160, 161, 197, 215
- Seeger, D. R., 6 (see Angier), 13 (see Angier; Boothe; Hultquist; Waller),
14 (see Waller), 15 (see Waller), 16
(see Waller), 17, 19 (see Angier), 22,
23, 24, 25, 85 (see Angier), 93, 198
(see Angier), 201 (see Waller), 214,
223
- Seegers, W. H., 34, 52, 53
- Seeler, A. O., 42, 51, 52, 71, 75, 83, 93,
97, 98, 99
- Selkurt, E. E., 287, 326
- Sellman, R., 181, 217
- Sells, R. L., 43, 52
- Selye, H., 305 (see Kutz), 324
- Semb, J., 6 (see Angier; Mowat), 7 (see Stokstad), 8 (see Mowat; Stokstad),
9 (see Hutchings; Stokstad), 11 (see Mowat), 13 (see Angier; Boothe;
Hultquist; Waller), 14 (see Waller),
15 (see Boothe; Hutchings; Mowat;
Waller), 16 (see Hutchings; Waller),
19 (see Angier; Boothe; Mowat), 21
(see Hutchings), 22, 23, 24, 25, 85
(see Angier), 93, 198 (see Angier;
Hutchings), 201 (see Waller), 214,
217, 223
- Semmons, E. M., 115, 160
- Service, W. C., 79, 99
- Sevag, M. G., 191, 192, 193, 221
- Seyfried, H., 87, 97
- Shannon, J. A., 287, 326
- Shapiro, S., 41, 53, 72, 99
- Shaw, J. H., 56 (see Schweigert), 60, 67
- Sheard, C., 123 (see Steffens), 161
- Sheely, R. F., 44, 53
- Shemiakin, M. M., 34, 53
- Sheppard, R., 316 (see Ingle), 324
- Sherman, H., 41 (see Lockhart), 51
- Sherwood, R. M., 86 (see Melass), 97
- Shettles, L. B., 44, 50, 150 (see Holt), 158
- Shimkin, M. B., 92, 99
- Shimotori, N., 90 (see Morgan), 98
- Shipley, R. A., 290, 326
- Shive, W., 183, 189, 216, 220, 221
- Shukers, C. F., 17 (see Day), 23
- Shute, E. V., 92, 100, 149 (see Vogelsang),
161
- Shute, W. E., 149 (see Vogelsang), 161
- Shvezov, J. B., 34 (see Shemiakin), 53
- Sickels, J. P., 6 (see Angier), 13 (see Angier; Boothe; Hultquist; Waller),
14 (see Waller), 15 (see Waller), 16
(see Waller), 19 (see Angier), 22, 23,
25, 85 (see Angier), 93, 198 (see Angier),
201 (see Waller), 214, 223
- Sicular, A., 125 (see Rapaport), 160
- Siegel, L., 195 (ref. 270), 221
- Siegwart, A., 9 (see Karrer), 23
- Sievers, R. F., 87, 99
- Silber, R. H., 71 (see Porter; Seeler), 74,
98, 99
- Silver, R. H., 42 (see Seeler), 52
- Silverman, M., 166 (ref. 271), 167, 221
- Silvette, H., 280, 284, 288, 298, 299, 306
(see Britton), 322, 326
- Sims, E. S., 21, 24
- Sinclair, H. M., 104, 110, 115, 117, 118,
120, 133, 139, 142, 149, 153, 160, 161
- Singal, S. A., 140 (see Sydenstricker), 161

- Singer, J. H., 30, 32, 52
Singh, K., 166 (ref. 3, 272), 174 (ref. 3, 272), 178, 184 (ref. 3, 272), 187, 189 (ref. 272), 190, 198 (see Agarwal), 199, 214, 221
Sjorgren, B., 216
Skeggs, H. R., 190, 193 (see Spink), 200, 202, 222, 223
Skoog, F., 239, 274
Sloane, N. H., 4, 23, 85 (see Day), 95, 201 (see Hutchings), 217
Slobodkin, N. H., 4 (see Hutchings), 23
Smadel, J. E., 82 (see Hamilton, H. L.), 95
Smedley-MacLean, I., 206, 221
Smith, A. H., 281, 322, 324, 326
Smith, A. M., 313 (see Kinsell), 324
Smith, B. F., 72 (see Williams, R. D.), 100
Smith, C. A., 132, 161
Smith, C. B., 80, 99
Smith, C. C., 40, 53, 93, 99
Smith, D. E., 288, 324, 326
Smith, D. G., 74, 95
Smith, H. D., 83 (see Harned), 95
Smith, H. W., 287, 326
Smith, J. K., 60 (see Anderson), 66
Smith, J. M., 148, 159
Smith, J. M., Jr., 6 (see Angier), 13 (see Angier; Boothe; Hultquist; Waller), 14 (see Waller), 15 (see Waller), 16 (see Waller), 17 (see Seeger), 19 (see Angier), 22, 23, 24, 25, 85 (see Angier), 93, 198 (see Angier), 201 (see Waller), 214, 223
Smith, M. B., 210 (see Bauernfeind), 211 (ref. 20), 214
Smith, P. K., 82, 99, 281, 283, 287 (see Schwartz), 308 (see Winkler), 324, 326, 327
Snavelly, J. R., 72 (see Turner), 99
Snell, A. M., 28, 44 (see Clark, C. L.), 49, 317 (see Wilder), 327
Snell, E. E., 183, 194, 195, 196, 216, 220, 221, 264, 274
Snow, A. G., Jr., 242, 271
Snyder, J. C., 82, 98, 99, 100
Soffer, L. J., 280 (see Harrop), 284 (see Harrop), 287 (see Harrop), 316 (see Harrop), 323
Soloway, S., 35 (see Oppenheimer), 52
Somers, G. F., 247, 274
Somkin, E., 79, 97
Somogyi, J. C., 283, 326
Søndergaard, E., 34, 49
Sonne, J. C., 267, 274
Soskin, S., 128 (see Rosenbaum), 160, 306, 322
Souter, A. W., 45, 50
Southwick, P. L., 5 (see Wolf), 12 (see Wolf), 25, 202 (see Wolf), 223
Spangler, J. M., 83 (see du Vigneaud), 95
Sperber, E., 172, 173, 221
Spero, L., 34 (see Field), 50
Spickard, V. W., 77 (see Durand), 95
Spies, T. D., 71, 73, 76, 78, 80, 81, 85 (see Vilter, C. F.), 95, 99, 100, 201, 222
Spiesman, I. G., 89, 99
Spillane, J. D., 122, 147, 161
Spinella, J. R., 127 (see Cogswell), 158
Spink, W. W., 36 (see Armstrong, W. D.), 46 (see Armstrong, W. D.), 48, 93 (see Armstrong), 94, 193, 222
Spoor, H. J., 305, 312, 324, 326
Sproul, E., 44, 53
Sreenivasaya, M., 206, 220
Stahl, J., 280 (see Loeb), 287 (see Loeb), 325
Stannus, H. S., 137, 144, 161
Stark, W. H., 175 (see Novak), 219
Steck, I. E., 90 (see Reed), 91 (see Reed), 98
Steenbock, H., 205 (ref. 221), 209, 214, 217, 220
Stefanini, M., 20, 40, 42, 52
Steffens, L. F., 123, 161
Steiger, M., 314, 326
Stein, H. B., 45, 53
Stein, L., 295, 326
Stein, W., 76, 99
Steinbach, H. B., 303, 326
Steinberg, C. L., 76, 99
Steinberg, R. A., 176, 222
Steven, D. M., 122, 123, 161
Stevens, J. R., 194 (see Stillner), 222
Stewart, G. N., 279, 280, 321, 326
Stickney, J. C., 283, 326
Stiles, H. R., 180, 222
Stillner, E. T., 194, 222

- Stodola, F. A., 204 (see Anderson, R. J.), 214
- Stoerk, H. C., 80, 99
- Stokes, J. L., 4 (see Keresztesy), 23, 189, 191, 194, 195, 196, 198, 200, 201, 217, 222
- Stokstad, E. L. R., 3, 4, 6 (see Angier; Mowat), 7, 8, 9, 11 (see Mowat), 13 (see Angier; Boothe; Hultquist; Waller), 14 (see Waller), 15, 16 (see Franklin; Hutchings; Waller), 19 (see Angier; Boothe; Mowat), 20, 21, 22, 23, 24, 25, 84, 85, 93, 95, 198 (see Angier; Hutchings), 201, 207, 214, 217, 223
- Stolovy, E., 183 (see Ryan), 191 (see Ryan), 221
- Stone, L., 210 (see Bauernfeind), 211 (ref. 19, 20), 214
- Stone, R. E., 78 (see Spies), 99
- Stoppelmann, Marie, 44, 53
- Stoutemyer, V. T., 260, 274
- Strachan, H., 122, 161
- Strauss, M. B., 280 (see Harrop), 281 (see Harrop), 323
- Streightoff, F., 193 (see Landy), 218
- Strong, F. M., 183 (see Snell, E. E.), 205 (ref. 130), 217, 221, 235 (see Woolley, D. S.), 275
- Struck, H. C., 90 (see Reed), 91 (see Reed), 98
- Stubbs, J. J., 166 (ref. 157), 169 (see Lewis, J. C.), 174 (ref. 157), 179 (see Lewis, J. C.), 182 (ref. 157), 189 (ref. 157), 190 (see Lewis, J. C.), 193 (see Lewis, J. C.), 194 (see Lewis, J. C.), 195 (ref. 157), 197 (see Lewis, J. C.), 218, 222
- SubbaRow, Y., 3, 4 (see Hutchings), 6 (see Angier; Mowat), 7 (see Stokstad), 8 (see Mowat; Stokstad), 9 (see Hutchings; Stokstad), 11 (see (see Mowat), 13 (see Angier; Boothe; Hultquist; Waller), 14 (see Waller), 15 (see Boothe; Hutchings; Mowat; Waller), 16 (see Hutchings; Waller), 19 (see Angier; Boothe; Mowat), 21 (see Hutchings), 22, 23, 24, 25, 85 (see Angier) 93, 198 (see Angier; Hutchings), 201 (see Hutchings; Waller), 214, 217, 223
- Sugiura, K., 80 (see Kensler), 83 (see du Vigneaud; Kensler), 95, 96
- Sukhorukov, K., 242, 274
- Sullivan, M., 138, 139, 161
- Sullivan, M. X., 30, 50
- Sullivan, R. A., 212, 222
- Sullivan, R. C., 284, 327
- Sullivan, W. R., 41 (see Link), 51
- Sulzberger, M. B., 87, 99
- Summerson, H., 36, 53
- Sure, B., 75, 76, 99, 116, 161
- Sutherland, I., 132, 161
- Sutton, W. R., 74 (see Youmans), 100
- Swaminathan, M., 166 (ref. 300), 184 (ref. 300), 188, 222
- Swank, R. L., 108, 160, 161
- Swartz, D. B., 257, 258, 274
- Sweet, L. K., 44, 52
- Swendseid, M. E., 21 (see Mims), 23
- Swingle, K. F., 235 (see Dorfman), 272
- Swingle, W. W., 280, 283, 289, 294, 298 (see Parkins), 300 (see Parkins), 314, 320, 325, 326, 327
- Sydenstricker, V. P., 78, 99, 122 (see Kruse), 127 (see Kruse), 137, 140, 142, 144, 145, 146, 159, 161
- Szent-Györgyi, A., 87, 88, 98, 99

T

- Taffel, M., 284 (see Darrow), 322
- Tage-Hansen, E., 44, 49
- Talbot, N. B., 313, 327
- Tandy, D., 193 (see Lemberg), 218
- Tanner, F. W., Jr., 174 (ref. 301-304), 179, 180, 182, 183, 184 (ref. 301), 189 (ref. 301), 195 (ref. 301), 210, 211 (ref. 301), 212, 222
- Tanret, C., 204, 222
- Tapia, E. W., 245 (see Cravioto), 246 (see Cravioto), 272
- Tarbell, D. S., 29, 53
- Tastaldi, H., 29, 53
- Tatum, E. L., 168, 191, 193, 222, 233, 261, 265, 266, 274
- Tauber, H., 261, 274
- Taylor, A., 80 (see Pollack), 98

- Taylor, A. R., 286 (see Parkins; Swingle),
 320 (see Swingle), 325, 326
 Taylor, B. L., 60 (see Howell), 66
 Taylor, E. C., Jr., 9 (see Cain; Mallette),
 17 (see Cain; Mallette), 22, 23
 Taylor, E. S., 44 (see Beck), 49
 Taylor, H. L., 127 (see Keys), 129, 159,
 161
 Taylor, J., 80 (see Pollack), 81, 98, 99
 Tchang, J. L., 209, 216
 Templeman, W. G., 257, 258, 259, 274
 Teply, L. J., 79 (see Krehl), 96
 Terroine, E. F., 206, 222
 Teyssiere, Y., 172, 222
 Thaddea, S., 300, 327
 Thatcher, J. S., 312, 313 (see Hartman),
 323, 324
 Thayer, S. A., 28 (see Doisy), 49, 208,
 219, 222, 313 (see Olson), 325
 Thaysen, A. C., 166 (ref. 314), 184 (ref.
 314), 222
 Therien, M., 87, 95
 Thimann, K. V., 260, 274, 275
 Thomas, B. G. H., 93 (see Ansbacher), 94
 Thomas, E. M., 206, 221
 Thomas, J. M., 195 (ref. 27), 215
 Thomas, P. E., 267 (see Fosse), 272
 Thompson, H. E., 71 (see Wyatt), 100
 Thompson, R. C., 166 (ref. 315), 167,
 168, 169, 182, 184 (ref. 315), 186,
 187, 189, 190, 194, 195 (ref. 315),
 197 (ref. 315), 198, 199, 200, 222
 Thorn, G. W., 313, 314, 315, 316, 318, 327
 Tice, J. W., 141 (see Adamson), 143
 (see Adamson), 145 (see Adamson),
 147 (see Adamson), 157
 Tierney, N. A., 82, 99
 Tilden, E. B., 47 (see Burrill), 49
 Tinker, M. A. H., 260, 274
 Tisdall, F. F., 79, 99, 127 (see Kruse), 131
 (see Ebbs), 133, 141 (see Adamson),
 143 (see Adamson), 145 (see Adam-
 son), 147 (see Adamson), 157, 158,
 159, 161
 Tishler, M., 9 (see Weijlard), 25, 29, 53
 Tittsler, R. P., 175, 222
 Tolman, L., 16, 17, 23
 Tomkins, F. S., 33 (see Ewing), 49
 Tönnis, B., 188, 217
 Tooke, T. B., Jr., 319, 327
 Torda, C., 93, 99
 Törnquist, R., 141 (see Frantzell), 158
 Totter, J. R., 4 (see Day), 21, 23, 24, 85
 (see Day), 95, 146, 147, 161, 201,
 222
 Trager, W., 83, 99
 Traube, W., 8, 24
 Trenner, N. R., 11, 19, 24, 32, 33, 53
 Trescher, J. H., 284 (see Harrop), 287
 (see Harrop), 316 (see Harrop), 323
 Tronsberg, G., 44, 53
 Truesdail, J. H., 181 (see Williams, R. J.),
 223
 Trufanov, A. V., 203, 222
 Truszkowski, R., 309, 316, 317, 327
 Tukey, H. B., 238, 258, 274
 Turner, E., 285, 302, 322
 Turner, R. H., 72, 99
 Turrell, F. M., 255, 257 (see Parker), 274
 Tuttle, L. C., 181, 218, 266 (see Lipmann),
 273
 Tuxford, A. W., 130, 161
 Tweedie, M. C. K., 157
- U**
- Uhlmann, R., 79, 99
 Uldall, C., 43, 52
 Ulmer, N. Z., 289 (see Joseph, S.), 324
 Umbreit, W. W., 189, 194 (see Lichstein),
 195 (see Bellamy), 196 (see Bel-
 lamy), 215, 218, 264, 265 (see
 Lichstein), 267, 272, 273, 275
 Underkofler, L. A., 174 (ref. 319), 183,
 222, 265, 275
 Unger, P. N., 72, 99
 Unna, K., 70, 74, 76, 77, 79, 80, 81, 94,
 98, 99, 100, 235, 275
 Unwin, C. H., 260, 274
 Urist, H., 17 (see Martin), 23
- V**
- Vail, D., 145, 146, 161
 Vallin, I., 172, 219
 Van Hoogenhuyze, C. J., 171 (see
 Eijkman), 216
 Vandenberg, J. M., 5 (see Wittle), 6 (see
 O'Dell), 20 (see Bird), 22, 24, 25, 34
 (see Seegers), 52

- Van Lanen, J. M., 166 (ref. 322), 169, 170, 171, 172, 173, 174 (ref. 301-304) 178, 179 (see Tanner), 180, 182 (see Tanner), 183 (see Tanner), 184 (ref. 301, 320), 185, 186, 187, 188, 189 (ref. 301), 195 (ref. 301), 210 (see Tanner), 211 (ref. 301), 212 (see Tanner), 222, 223
 Van Overbeek, J., 238, 255, 275
 Varco, R. H., 291 (see Visscher), 327
 Varnadoe, N. B., 40, 52
 Vars, H. M., 280 (see Pfiffner), 289 (see Swingle), 294 (see Swingle), 320 (see Swingle), 326, 327
 Vechet, J., 206 (see Terroine), 222
 Vedder, A., 89, 100
 Veldman, H., 173, 223
 Venndt, H., 43, 53
 Vennesland, B., 261, 262, 263, 272, 275
 Verdoo-Roe, C. M., 115 (see McCance), 159
 Verzár, F., 283, 295, 296, 301, 324, 325, 326, 327
 Victor, J., 125, 161
 du Vigneaud, V., 83, 95, 96, 188, 190, 191, 215, 219, 223, 266, 275
 Vilter, C. F., 85, 100
 Vilter, R. W., 85 (see Vilter, C. F.), 100
 Vinson, L. J., 166 (ref. 326), 170, 223
 Virtanen, A. I., 265, 275
 Visscher, M. B., 290, 291, 292, 322, 324, 327
 Vivino, A. E., 245, 275
 Vivino, J. J., 193 (see Spink), 222
 Vogelsang, A., 92, 100
 Vogelsang, A. B., 149, 161
 Vogt-Möller, P., 91, 100
 Vojnovich, C., 174 (ref. 304), 222
 Vonesch, E. E., 29, 53
 Vorhaus, M. G., 77, 100
 Votiak, E. G., 71, 100
 Vyvyan, M. C., 240, 275
- W**
- Wade, N. J., 313 (see Olson), 325
 Wadsworth, C., 83 (see Kensler), 96
 Wagner, K. H., 123, 161, 169 (see Scheunert), 221
 Waisman, H. A., 62 (see Cooperman), 63 (see Cooperman), 64 (see Cooperman), 66, 184 (ref. 327), 223
 Wakerlin, G. E., 35, 51
 Wakim, K. G., 40, 53
 Wald, G., 77, 96, 122, 123, 161
 Waldenström, J., 141 (see Frantzell), 158
 Walker, E., 204, 206, 207, 219
 Walker, S. A., 43 (see Sells), 52
 Walker, S. E., 42, 53, 89, 100
 Waller, C. W., 6 (see Angier; Mowat), 7 (see Stokstad), 8 (see Mowat; Stokstad), 9 (see Hutchings; Stokstad), 11 (see Mowat), 13, 14, 15, 16, 19 (see Angier; Boothe; Mowat), 21 (see Hutchings), 22, 23, 24, 25, 85 (see Angier), 93, 198 (see Angier; Hutchings), 201, 214, 217, 223
 Walton, M. T., 175, 223
 Wang, Y. L., 42, 51
 Wangerin, D. M., 150 (see Holt), 158
 Warburg, O., 226, 275
 Ward, A. H., 247, 275
 Ward, J. L., 36, 48
 Ware, A. G., 34, 53
 • Waring, W. S., 179, 223
 Warner, G. C., 259 (see Went), 260, 275
 Waterman, R. E., 77 (see Vorhaus), 100
 Watson, C. J., 85, 100
 Weaver, H. M., 56, 67
 Weese, H., 75, 96
 Weevers, Th., 250, 275
 Weigand, C. G., 80, 98
 Weijlard, J., 9, 25
 Weiland, P., 204, 223
 Weinstein, A., 284, 316 (see Harrop), 323
 Weinstock, H. H., Jr., 223
 Weisblat, D. I., 9, 24
 Weiss, S., 71, 73, 100
 Weissman, N., 74 (see Ferrebee), 95
 Weisz-Tabori, E., 262, 273
 Welch, A. D., 85, 96, 97, 100, 202 (see Wright), 223
 Wells, B. B., 297, 300, 313, 316, 327
 Wells, J. J., 80, 100
 Wenck, P. R., 204, 205 (ref. 332), 223
 Went, F. W., 240, 241, 250, 259, 260, 270, 275
 Wenzel, J. W., 314 (see Cleghorn), 322

- Werkman, C. H., 166 (ref. 271), 167, 179, 221, 223
 Wertheimer, E., 295, 326
 West, P. M., 83, 100, 167, 223, 255, 275
 Westenbrink, H. G. K., 173, 223
 Wetzol, N. C., 131, 161
 Weyer, E. R., 175, 223
 Wheatley, D. P., 46, 53
 Wheeler, A. H., 62, 67
 Whipple, D. V., 74, 100
 White, A. G. C., 169, 223
 White, C., 141, 161
 White, P. R., 227, 228, 229, 230, 238, 239, 275
 Whitmore, F. C., 204 (see Zook), 205 (ref. 360), 224
 Whitmore, W. H., 77, 100
 Whittenberger, J., 127 (see Egaña), 158
 Whittier, F. O., 175, 222
 Wick, A. N., 296, 322
 Wickerham, L. G., 180, 223
 Widdowson, E. M., 115, 159, 161
 Widenbauer, F., 86, 100
 Wiehl, D. G., 127 (see Borsook), 157
 Wieland, T., 183, 217, 223
 Wikén, B., 257, 258, 273
 Wilde, W. S., 283 (see Fenn), 323
 Wilder, R. M., 72 (see Williams, R. D.), 100, 127 (see Kruse), 141 (see Adamson), 143 (see Adamson), 145 (see Adamson), 147 (see Adamson; Williams, R. D.), 157, 159, 162, 317, 322, 327
 Wildman, S. G., 262, 268, 271
 Wiles, H. O., 86 (see Maurer), 97
 Wiley, F. H., 284, 327
 Wiley, L. L., 284, 327
 Wilkening, M. C., 59 (see Schweigert), 67
 Wilkins, R. W., 71, 73, 100
 Wilkinson, J. F., 87, 100
 Wilkinson, P. B., 122, 162
 William, A., 256, 275
 Williams, H. L., 78, 100
 Williams, J. Rhys, 131, 162
 Williams, R. D., 72, 74, 97, 100, 147, 162
 Williams, R. H., 77, 100
 Williams, R. J., 80 (see Pollack), 98, 169, 181, 186, 187, 190, 192, 194, 196, 199, 216, 221, 223, 243, 264 (see Snell), 265, 272, 274, 275
 Williams, R. R., 73, 76, 77 (see Vorhaus), 100
 Williams, S. L., 195 (ref. 15), 214
 Williamson, R., 139, 162
 Willson, D. M., 317, 327
 Wilson, K., 186 (see Burkholder), 200 (see Burkholder), 201 (see Burkholder), 215
 Wilson, P. W., 167, 223
 Wilson, R. H., 88, 89, 100
 Wing, M., 41 (see Rapoport), 52
 Winkler, A. W., 283, 287 (see Schwartz), 308, 326, 327
 Winnick, T., 191, 217, 220, 223
 Winter, C. A., 315, 327
 Wirth, J. C., 170, 223
 Wiseman, R. D., 91 (see Kaufman), 96
 Wittle, E. L., 5, 25
 Witts, L. J., 86, 97
 Woglom, W. H., 83, 100
 Wolbach, S. B., 89, 100, 130, 162
 Wolf, D. E., 5, 12, 25, 202, 223
 Wolf, I. J., 91, 100
 Wolfe, J. K., 33 (see Hershberg), 50
 Wolff, H., 78 (see Karl), 93, 96, 99
 Wood, E. H., 283, 292, 294 (see Kottke), 295, 313, 322, 324, 327
 Wood, H. G., 168 (see Tatum), 222
 Wood, S., 194 (see Lyman), 218
 Woodhouse, W. W., Jr., 257, 258, 275
 Woods, D. D., 192, 223
 Woodward, C. R., Jr., 194 (see Stokes), 195 (see Stokes), 196 (see Stokes), 222
 Woolley, D. W., 37, 39, 40, 53, 60, 67, 116, 139, 162, 169, 173, 183, 197, 208, 217, 223, 235, 275
 Wortis, E., 125 (see Liebmann), 159
 Wortis, H., 125 (see Liebmann), 159
 Wright, E. J., 122, 162
 Wright, L. D., 85, 97, 100, 190, 193 (see Spink), 200, 202, 222, 223
 Wyatt, B. L., 71, 100
 Wynd, F. L., 259, 273

Y

- Yamasaki, I., 175, 176, 224
 Yannet, H., 285, 307, 322

Yeomans, A., 82, 99, 100

Yoshitome, W., 175, 224

Youmans, J. B., 74, 100, 117, 162

Young, C. M., 115, 162

Young, N. F., 80 (see Kensler), 96

Young, W. J., 187, 216

Z

Zacho, C. E., 88, 100

Zakharova, M. P., 33, 53

Zamecnik, P. C., 141 (see Adamson), 143
(see Adamson), 145 (see Adamson),
147 (see Adamson), 157

Zarafonetis, C. J. D., 82, 98, 99, 100

Zepplin, M., 60 (see Schweigert), 67

Zervas, L., 15, 22

Ziegler, W. M., 35, 52, 93, 99

Zillesson, F. O., 313 (see Kinsell), 324

Zilva, S. S., 88, 100

Zirplel, W., 205 (ref. 359), 209, 224

Zimmerman, P. W., 256, 257, 260, 272

Ziskin, D. E., 143, 162

Zook, H. D., 204, 205 (ref. 360), 224

Zopf, L. C., 256, 275

Zorkoczy, J., 206, 224

Zwemer, R. L., 280, 284, 309, 313, 317,
325, 327

Subject Index

A

- Abortion, vitamin E and, 91, 148
- 1-Acetoxy-2-methyl-4-naphthyl sodium phosphate,
 - vitamin K activity of, 28
- 1-Acetoxy-2-methyl-4-naphthyl sodium sulfate,
 - vitamin K activity of, 28
- Acetylcholine,
 - effect on distribution of potassium, 283
 - of vitamin K on synthesis of, 93
- Addison anemia, ascorbic acid and, 87
- choline and, 86
- Addison's disease,
 - diuresis and, 317
 - therapeutic effect of sodium salts in, 318, 319
 - of desoxycorticosterone acetate in, 318, 319
- Adenine, 226
 - biochemical activity in plants, 267, 268
 - biological conversion to hypoxanthine, 267
 - distribution in plants, 249, 270
 - growth activity for intact plants, 256
 - as leaf-growth factor, 240, 241, 256, 267, 269, 270
 - site of synthesis in plants, 269
- Adrenal cortex,
 - chemical nature of amorphous fraction, 313
 - effect of extract of, on sodium retention, 312, 319
- Adrenal insufficiency, *see also* Adrenalectomy
 - diagnosis of, 316, 317
 - symptoms of, 306-312
 - treatment of, 317-319
- Adrenalectomy, *see also* Adrenal insufficiency
 - composition of blood following, 279, 280
 - effect on distribution of electrolytes, 284, 285
 - of water, 284-286
 - on excretion of electrolytes, 287, 288
 - of water, 289, 290
 - on metabolism of electrolytes, 279, 280
 - on renal function, 288, 289
 - physiological effects of sodium chloride and, 295-305
 - retention of potassium and, 307-312
 - survival after, 279-281
- Adrenaline, antagonism to niacin, 78
- Adrenocortical hormones,
 - effect on metabolism of chloride, 312-321
 - of potassium, 312-321
 - of sodium, 312-321
- Aerobacter aerogenes*,
 - riboflavin content of, 182
 - synthesis of folic acid by, 200
 - of niacin by, 186, 187
 - of thiamine by, 168
 - thiamine content of, 166
- Alanine, biosynthesis of pyridoxine and, 195, 196
- β -Alanine, biosynthesis of pantothenic acid from, 183
- requirements for, 265
- Algae,
 - effect of menadione on, 37, 38
 - of phthiocol on, 37, 38
 - as source of ascorbic acid, 203
- Allantoic acid, occurrence in plant tissues, 267
- Allantoin, occurrence in plant tissues, 267
- Alopecia,
 - inositol and, 139
 - vitamins and, 139
- Amino acids,
 - diet deficient in, and serum protein, 118
 - effect on eyes, 144, 146, 147
 - erythropoiesis and, 149

- as leaf-growth factors, 240
- spermatogenesis and, 150
- p-Aminobenzoic acid, 164, 191, 192, 226
- antagonism to sulfonamides, 71, 81 193
- biochemical role of, 192
- content of yeasts, 193
- deficiency of, and greying of fur, 139
- detoxifying action on arsenicals, 82
- distribution in plants, 244, 250
- folic acid and, 192, 193
- requirement of hamster, 63
- of rats, 58
- synergism with salicylates, 82
- synthesis by microorganisms, 192
- in roots, 237, 250
- sulfonamides and, 237
- therapeutic value in rickettsial disease, 82
- toxic effects of, 82
- toxicity of, 81, 82
- 2-Amino-4-hydroxy-6-carboxy-7-methylpteridine,
- synthesis of, 6
- 2-Amino-4-hydroxy-6-methylpteridine,
- 8, 10, 11
- synthesis of, 9
- 2-Amino-4-hydroxypteridine, 8
- 2-Amino-4-hydroxypteridine-6-carboxylic acid, 6, 10, 11
- 2-Amino-4-hydroxypteridine-6 (or 7)-carboxylic acid, 8
- synthesis of, 8
- 4-Amino-2-methyl-1-naphthol, 31
- biological conversion to menadione, 34
- determination, 31, 32
- hydrochloride of, antibiotic properties of, 36
- 2-Amino-5-methylpyrazine-3-carboxylic acid, 9
- l*- α -Amino-4-methyl-5-thiazolepropionic acid, biological conversion to 4-methyl-5- β -hydroxyethylthiazole, 171
- β -Aminopyridine, effect on root growth, 235
- Anaphylaxis, rutin and, 89
- Anemia,
- hypochromic,
- deficiency symptoms in, 149
- pernicious,
- deficiency symptoms in, 141
- therapeutic effect of choline in, 86
- of pteroylglutamic acid in, 84, 85
- Angiopathia retinalis juvenalis, see Eales' disease
- Antibiotics, effect on biosynthesis of thiamine, 74
- Aporhizopterin,
- absorption spectrum of, 11
- activity of, 11
- empirical formula, 11
- vitamin B₆ and, 12
- Arachidonic acid,
- effect of deficiency of, on skin, 139
- Arecoline, effect on root growth, 235
- Arsenicals, detoxification of pentavalent, by p-aminobenzoic acid, 82
- Ascorbic acid, 202-204
- capillary fragility and, 125
- connective tissues and, 148
- diuretic activity of, 86
- Eales' disease and, 110
- effect on fracture repair, 87
- on methemoglobinemia, 87
- on riboflavin synthesis, 181
- on wound healing, 87, 148
- folliculosis and, 139
- gingivitis and, 142, 143
- hemorrhages and, 140
- ideal nutriture of, 112
- occurrence in blood, 117, 118, 154
- in microorganisms, 202, 203
- pregnancy and, 143
- resistance to low temperature and, 88
- synthesis by microorganisms, 203, 213
- by vegetal embryos, 238
- teeth and, 143
- therapeutic value in Addison anemia, 87
- in allergic conditions, 71, 88
- toxicity of, 86
- urinary excretion of, 121, 154
- Ashbya gossypii*, riboflavin
- content of, 174
- riboflavin synthesis by, 174, 179, 180
- Asparagin, effect on niacin biosynthesis, 186
- Aspergillus niger*,
- riboflavin content of, 174

- synthesis of p-aminobenzoic acid by, 193
 - of ascorbic acid by, 203, 213
 - of riboflavin by, 180, 181
- Asphyxia, effect on potassium distribution, 283
- Asthma, ascorbic acid and, 88
- Atabrine, antagonism of pyridoxine to, 71
- Avidin, effect on biosynthesis
 - of biotin, 83, 190
- Auxin, see also Indoleacetic acid, callus formation and, 239
- Azotobacter chroococcum*, niacin content of, 184, 185

B

- Bacillus brevis*,
 - as source of vitamin K₂, 29
- Bacillus butyricus*, ergosterol content of, 204, 205
- Bacillus mesentericus*, niacin synthesis by, 186
- Bacillus prodigiosus*, synthesis of ascorbic acid by, 203
- Bacillus vulgatus*, niacin synthesis by, 186
- Bacteria,
 - as source of vitamins B, 167
 - thiamine content of, 166
 - synthesis by, 167-169
- Beans, vitamin B content of, 246
- Beets, vitamin B content of, 243
- Benzoic acid, effect on root growth, 235
- Beriberi, thiamine and, 71, 77, 149
- Biotin, 164, 165
 - analogues, biosynthesis of, 190, 191
 - growth activity for microorganisms, 191
 - biochemical role of, 188, 189
 - biosynthesis of, 237, 266
 - avidin and, 83
 - cysteine and, 266
 - pimelic acid and, 191, 266
 - sulfonamides and, 83
 - content of microorganisms, 189, 190
 - of tumor tissue, 83
 - deficiency of,
 - alopecia and, 139
 - resistance to bacterial infection and, 83

- skin and, 139, 140
- desthiobiotin and, 266
- distribution in plants, 242, 243, 245
- effect of light on synthesis of, in germinating seedlings, 252, 253, 269
 - on vegetal embryo growth, 269, 270
- enzymes and, 267
- growth activity for cuttings, 260
- isolation of, 188
- pharmacological properties, 83
- physiological role of, 83
- procarcinogenic activity of, 83
- requirements of hamster, 63
 - of rats, 237
- site of synthesis in plants, 250, 252, 253, 266, 269
- toxicity of, 83
- Blood,
 - composition of, 118
 - adrenalectomy and, 279, 280
 - effect of vitamin K on serum anti-thrombin, 35
 - vitamins in, 117-120, 152, 154
- Bones,
 - ascorbic acid and repair of fractures, 87
- Butanol,
 - riboflavin in fermentation residues of, 175-178

C

- Caffeine, distribution in plant, 249, 250
- Calciferol, see Vitamin D₂
- Calcium,
 - connective tissues and, 148
 - effect of dietary, on serum, 120
 - eyes and, 144, 147
 - nails and, 141
 - teeth and, 143
- Callus, auxin and formation of, 239
- vitamins B and formation of, 230
- Camphor, effect on biosynthesis of niacin, 187
- Candida guilliermondia*
 - niacin content of, 185
 - riboflavin synthesis by, 173, 179
- Capillary fragility,
 - ascorbic acid and, 125
 - measurement of, 124

- Carbohydrate metabolism, adrenalectomy and effect of sodium chloride on, 298-301
- Carbon dioxide, effect on distribution of potassium, 283
- Carboxylases, occurrence in plants, 261
thiamine and, 262
- 2-Carboxy-3-methylchromone, vitamin K activity of, 29
- Cardiac glucosides,
adrenocortical hormones and, 313
effect on plasma potassium, 313
- Caries, dental,
in the cotton rat, 60, 61
hamster, 62
fluorine and, 144
vitamin K and, 46, 47, 93
- β -Carotene,
biosynthesis of, 209, 213
- Carotenoids,
in blood serum, 54
effect of diet on, in blood, 119
of malnutrition on utilization of, 124
metabolism of, 124
synthesis by microorganisms, 209, 213
- Carotinemia, manifestations of, 89, 90
- Carrots, vitamin B content of, 243
- Casein, effect of dietary, on niacin requirement of cotton rat, 58
- Cereals, vitamin B content of, 247
- Cheese, vitamin content of, 212
- Chickens,
antianemic activity of vitamin B₆ conjugate for, 3
of yeast and yeast extracts for, 2
growth activity of pteroylglutamic acid compounds for, 3, 4, 18, 19, 20
- Chloride,
adrenalectomy and distribution of, 279, 280, 284
and excretion of, 287, 288
content of muscle, 282
of tissues, 282
effect of adrenocortical hormones on metabolism of, 312-321
retention of, 281
transfer through intestinal wall, 290-292
- Choline, 226
eyes and, 144
methionine and biosynthesis of, 85
requirement of hamster, 63
of rats, 58
therapeutic effect in anemias, 86
toxic effects of, 86
toxicity of, 85
- Choline esterase, inhibitory effect of thiamine on formation of, 77
- Chronaxie, vitamins and, 125
- Citrus fruits, vitamin B content of, 246
- Clostridium acetobutylicum*,
p-aminobenzoic acid as growth factor for, 192
biosynthesis of riboflavin by, 173, 175-178
iron and, 177, 178
- Cobalt,
erythropoiesis and, 149
- Cocarcboxylase,
effect of diet on, in blood, 119
- Codecarboxylase, biological conversion of pyridoxamine to, 195, 196
- Coenzyme A,
occurrence in microorganisms, 181
pantothenic acid and, 181, 183, 266
- Cold, ascorbic acid and resistance to, 88
- Copper,
erythropoiesis and, 149
nervous system and, 147
- Coramine (N-diethylniacinamide), effect on root growth, 235
- Corn stigma,
as source of vitamin K₃, 29
- Corticosterone,
effect on excretion of potassium, 316
on retention of chloride, 316
of sodium, 316
- Corynebacterium* sp.
as source of fermentation *L. casei* factor, 4
- Cotton rat,
p-aminobenzoic acid requirement of, 58
choline requirement of, 58
diet and dental caries in, 60, 61
and lactation in, 61
and reproduction in, 61
dietary tryptophan and glucoascorbic acid requirement of, 60
and niacin requirement of, 59

effect of dextrin on, 60
of starch on, 60
growth of,
 inositol and, 58
 liver extract and, 58
nutritional requirements of, 56-61
purified diet suitable for growth studies
 with, 57
susceptibility to virus diseases, 56
vitamin B requirements of, 56
 C requirements of, 58
Cozymase, niacin and, 263, 269
Cricetus auratus, see Hamster
Cuttings,
 growth activity of pyridoxine for, 260
 of thiamine for, 259, 260
2-(3-Cyclohexylpropyl)-3-hydroxy-1,4-
 naphthoquinone (S 5090),
 toxic effects of, 40
 menadione and, 40, 93
 vitamin K₁ and, 40, 93
Cysteine, biosynthesis of biotin and, 266
Cytochrome, 263

D

2-[3-(Decahydro-2-naphthyl)propyl]-
 3-hydroxy-1,4-naphthoquinone,
 toxic effects of, 40
 menadione and, 40, 41
 vitamin K, and, 40, 41
7-Dehydrosterol, activated, see Vitamin
 D₃
Desiminorhizopterin, 12
Desoxycorticosterone,
 chemical structure and physiological
 activity, 321
 effect on metabolism of chloride, 314,
 321
 of water, 314
 on retention of potassium, 315, 316,
 319
 of sodium, 314, 315, 319
 therapeutic effect in Addison's disease,
 318, 319
Desoxyypyridoxine, effect on urinary
 excretion of xanthurenic acid, 71
Desthiobiotin,
 biosynthesis of, 191
 biotin and, 266

growth activity for microorganisms,
 191
Diabetes mellitus, thiamine and, 77
2,4-Diamino-6,7-diphenylpyrimido
 (4,5-b)pyrazine,
 antagonism to pteroylglutamic acid, 18
2,4-Diaminophenanthro(9,10-e)pyri-
 mido(4,5-b)pyrazine,
 antagonism to pteroylglutamic acid, 18
 synergism with sulfathiazole, 18
N-[4-[(2,4-Diamino-6-pteridyl)methyl]-
 amino]-benzoyl]-glutamic acid,
 antagonism to pteroylglutamic acid, 17
 structure of, 17
Diaphorase, occurrence in plants, 262
2,3-Dichloro-1,4-naphthoquinone, antag-
 onism to vitamin K, 40, 208
Dicumarol [3,3'-methylene-bis (4-hydro-
 xycoumarin)],
 antagonism of 2-methyl-4-hydroxy-
 coumarin to, 40
 to vitamin K, 39, 40, 93
 to vitamin K₁, 40, 93
 toxic effects of, 40
Dihydroxystearic acid,
 inhibitory effect on intestinal vitamin
 K synthesis, 41
Dihydrovitamin B₆, 6
 absorption spectrum of, 6
p-Dimethylaminoazobenzene, antag-
 onism of riboflavin to, 80
Dinicotinic acid, effect on root growth,
 235

E

Eales' disease,
 ascorbic acid and, 110
 vitamin A and, 111
Edema, nutritional, 149
Enzymes,
 of higher plants, 263
 proteolytic,
 vitamin K and, 35
 vitamins B and, 261-265
Epinephrine, effect on distribution of
 potassium, 283
Eriodictyol,
 toxicity of, 88

- Eremothecium ashbyii*,
 riboflavin content of, 174
 synthesis by, 174, 179
- Ergosterol,
 content of microorganisms, 205
 isolation of, 204
 sources of, 204
 synthesis by yeasts, 206, 207
- Ergot, as source of ergosterol, 204
- Ertron, see also Vitamin D
 toxic effects of, 91
- Erythropoiesis,
 amino acids and, 149
 minerals and, 149
 pteroylglutamic acid and, 149
 sodium chloride and, 305, 306
 vitamins and, 149
- Escherichia coli*, 267
 synthesis of pteroylglutamic acid by, 18
 of thiamine by, 168
 vitamin K and, 20, 37, 40
- Estradiol,
 effect on metabolism of water, 313
 on retention of chloride, 313
 of sodium, 313
- Estrogen(s),
 effect on gums, 143
 on metabolism of electrolytes, 313
 of water, 313
 as vegetal embryo growth factor, 238
- Estrone, as vegetal embryo growth factor, 238
- Estrous cycle, sodium chloride and, 305
- 3,3'-Ethylidene-bis (4-hydroxycoumarin), antagonism to vitamin K, 39
- Ethyl nicotinylacetate, effect on root growth, 235
- Eyes, effect of amino acids on, 144, 146, 147
 of choline on, 144
 of vitamins on, 121-124, 144-146
- metabolism,
 adrenalectomy and effect of sodium chloride on, 296, 301
- Fermentation *L. casei* factor, 1
 activity of, 4
 chemical composition, 4, 11
 constitution of, 15
 degradation of, 7-11
 identity with pteroylglutamic acid, 14, 15
 isolation of, 4
 solubility of, 4
 source of, 4
 synthesis of, 15
 therapeutic effect in vitamin M deficiency, 4
- Fermentation residues, vitamins B in, 210-212
- Fibrinogen, clotting of, 33
- Flavoproteins, occurrence in plants, 262, 263
 riboflavin and, 262
- Fluorides, effect on biosynthesis of p-aminobenzoic acid, 194
- Fluorine,
 effect on teeth, 143, 144
 on thyroid gland, 147
- Folic acid (see also Liver *L. casei* factor, Pteroylglutamic acid), 164, 165, 197 ff., 226
 p-aminobenzoic acid and, 192, 193
 content of microorganisms, 199
 distribution in plants, 242, 243, 245
 light and synthesis of, in germinating seedlings, 252, 253, 269
 site of synthesis in plants, 269
 synthesis from precursors, 201, 202
 by microorganisms, 200
- Fungi, as source of ascorbic acid, 202, 203
 thiamine synthesis by, 169, 170

G

- F
- Factor R, nature of, 20
- Factor U, nature of, 20
- Fat(s),
 animal, as source of vitamin D, 90
 effect of dietary, on biosynthesis of thiamine, 74
- Glucosascorbic acid,
 effect on cotton rat, 60
- Glucose,
 effect on distribution of chloride, 285
 of potassium, 283
 of sodium, 285
- Glycine, growth activity for roots, 229

- Growth,
 effect of nutrition on, 129 ff.
Guanine,
 biological conversion to xanthine, 267
 distribution in plants, 249, 250

H

- Hair,
 effect of vitamin deficiencies on, 139
Hamster,
 choline requirement of, 63
 dental caries in, 62
 inositol requirement of, 63
 nutritional requirements of, 61-65
 purified diets for growth studies with,
 62
 susceptibility to tubercle bacilli infec-
 tions, 62
 to virus diseases, 62
Hemopoietic factor,
 deficiency symptoms, produced by, 141
Hemorrhages, see also Capillary fragility
 ascorbic acid and, 140
 vitamin K and, 45
Hesperidin,
 toxicity of, 88
Hydrodicumarol [3,3'-methylene-bis (3,4-
 dihydro-4-hydroxycoumarin)], anti-
 coagulant effect of, 39
Hydroxyanthranilic acid, as intermediate
 in niacin biosynthesis, 186
17-Hydroxycorticosterone, effect on ex-
 cretion of potassium, 316
Hyperprothrombinemia, due to vitamin
 K administration, 34
Hypertension,
 effect of vitamin K on, 35, 93
Hyperthyroidism,
 thiamine and, 77
Hypoprothrombinemia,
 idiopathic, 43
 of the newborn, 43
 of pancreatic origin, 44
 vitamin K and, 38-44
Hypoxanthine,
 biological conversion to xanthine, 267
 as leaf-growth factor, 240, 269, 270
 as metabolite of adenine, 267
 occurrence in leaves, 249
 site of synthesis in plant, 269

I

- Indoleacetic acid, see also Auxins
 effect of combined application of
 thiamine and, on roots, 259 ff.
 on root development, 259, 264
 as growth factor for callus tissue, 239
Inhibition index(es), 16
 for pteroylglutamic acid inhibitors, 17
Inositol, 196, 226, 270
 absorption by yeast, 197
 content of microorganisms, 197
 deficiency,
 alopecia and, 139
 distribution in plants, 243, 245
 light and synthesis of, in germinating
 seedlings, 252, 253, 269
 requirement of hamster, 63
 of rats, 58
Intestinal wall,
 transfer of electrolytes through, 290-
 292
 of water through, 290-292
Iodine,
 thyroid gland and, 147
Iodinin,
 antagonism of naphthoquinones to, 36
Iron,
 effect on biosynthesis of p-amino-
 benzoic acid, 193
 of carotenoid, 209
 of folic acid, 199
 of inositol, 197
 of niacin, 186
 of pyridoxine, 196
 of riboflavin, 177-180, 214
 erythropoiesis and, 149
 nails and, 141
 tongue and, 141
Isonicotinic acid,
 effect on root growth, 235
Isoxanthopterine, 202

J

- Jaundice,
 vitamin K and, 43, 44

K

- Kynurenine, as intermediate in bio-
 synthesis of niacin, 186, 262

L

- Lactation**,
 dietary requirements for,
 in cotton rat, 61
 in hamster, 64, 65
- Lactobacillus arabinosus*,
 synthesis of pteroylglutamic acid by, 18
- Lactobacillus casei*, 5, 7
 growth activity of liver *L. casei* factor
 for, 3, 18
 of pteric acid for, 18
 of vitamin B₆ conjugate for, 2
 rhizopterin and, 4, 5, 18, 19
- Leaf-growth factors, 239-241, 269
 assay of, 240
- Leaves,
 growth factors for, 239-241, 269
 inactivation of thiamine in, 262
 occurrence of purines in, 249, 250
 synthesis of vitamins B in, 248, 249,
 269
- Leucocytes,
 content of water-soluble vitamins in,
 117
- Leucopterin, 202
- Light, effect on vitamin B synthesis in
 germinating seeds, 251, 252, 253, 269
- Linoleic acid,
 deficiency
 effect on skin, 139
- Lipositols, 196
- Liver,
 diagnostic value of vitamin K in dis-
 eases of, 45, 72
 effect of dicumarol on, 40
 of preparations of, on tongue, 141
 extract,
 effect on growth of cotton rat, 58, 60
- Liver *L. casei* factor (N-[4-[(2-amino-4-
 hydroxy-6-pteridyl) methyl] amino]
 benzoyl]-l-(+)-glutamic acid), see
 also Folic acid, Pteroylglutamic acid
 absorption spectrum of, 3
 growth activity of, 3
 identity with pteroylglutamic acid, 14
 isolation of, 3
 sources of, 3
 structure of, 11
 synthesis of, 13, 14

Lysine,
 diets deficient in, and serum protein,
 118

M

- Magnesium,
 deficiency symptoms, 139, 143
- Manganese,
 connective tissues and, 148
 effect on toxic effects of thiamine, 76
- Melanin, adrenalectomy and metabolism
 of, 303
- Menadione (2-methyl-1,4-naphthoquin-
 one), see also vitamin K
 administration to blood donors, 44
 antagonism to phthiocol derivatives,
 40, 41
 to sulfaquinoxaline, 42, 71
 antibacterial activity of, 93
 biological conversion of vitamin K
 derivatives to, 34
 bisulfite, 28, 34
 effect on algae, 37, 38
 on blood pressure, 35
 on mitosis, 38
 on tissue metabolism, 36
 hyperprothrombinemia and, 34
 lipotropic action of, 35
 methemoglobin formation due to, 36
 toxic effects of, 92, 93
 toxicity of, 92, 93
- Methionine, biosynthesis of choline and,
 85
- 2-Methyl-1-amino-4-naphthol hydro-
 chloride,
 daily requirement of the newborn, 43
- 3-Methylchromone, vitamin K activity
 of, 29
- 7-Methylfolic acid, antagonism to pteroyl-
 glutamic acid, 16, 17
 synthesis of, 16
- 2-Methyl-3-hydroxy-4-carboxy-5-hydrox-
 ymethylpyridine lactone,
 effect on utilization of glutamic acid
 compounds by chicks, 20
- 4-Methyl-5- β -hydroxyethylthiazole,
 biological conversion of l- α -amino-4-
 methyl-5-thiazolepropionic acid to,
 171
 biosynthesis of thiamine from, 171

- 2-Methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine lactone,
effect on utilization of pteroylglutamic acid compounds by chicks, 20
- 4-(3'-Methyl-4'-hydroxynaphthylazo)-benzenesulfonamide,
vitamin K activity of, 28
- 2-Methyl-1,4-naphthohydroquinone diphosphate, biological conversion to menadione, 34
- 2-Methyl-1,4-naphthoquinone, see also Menadione
skin irritation caused by, 70
- 2-Methyl-1,4-naphthohydroquinone disuccinate,
daily requirement of the newborn, 43
- N'-Methylniacinamide,
urinary excretion, 121, 154
- 7-Methylpteroic acid,
antagonism to pteroylglutamic acid, 17
- 7-Methylpteroylaspartic acid, antagonism to pteroylglutamic acid, 17
- 7-Methylpteroyl-l-(+)-glutamic acid,
antagonism to pteroylglutamic acid, 17
- 2-Methyl-1-tetralone, biological conversion to menadione, 34
- β -4-Methylthiazole-alanine, biological conversion into the thiazole component of thiamine, 232
- 5-Methyl-4,7-thionaphthenequinone,
vitamin K activity of, 28
- Methylene-bis(hydroxy-1,4-naphthoquinone), antagonism to vitamin K, 39
- 2-(2-Methyloctyl)-3-hydroxy-1,4-naphthoquinone,
toxic effects of, 40
menadione and, 40, 41
vitamin K₁ and, 40, 41
- Microorganism, distribution of vitamins in, 164-214
synthesis of vitamins by, 164-214
- Mineral oil, vitamin K deficiency and, 42
- Mitosis,
effect of vitamin K on, 38
- Molds, thiamine content of, 166
thiamine synthesis by, 170
- Muscle(s),
chloride content of, 282
potassium and, 148, 282
sodium content of, 282
vitamins and, 148, 149
- Mycobacterium paratuberculosis*, vitamin K compounds and, 37
- Mycobacterium smegmatis*, riboflavin synthesis by, 178
- Mycobacterium tuberculosis*,
biosynthesis of p-aminobenzoic acid by, 192
of niacin by, 186
of riboflavin by, 178
as source of phthiocol, 207
- N**
- Nails,
effect of vitamin deficiencies on, 139
- 1,4-Naphthoquinone,
antibacterial activity of, 93
- Naringin, toxicity of, 88
- Neurospora*,
biosynthesis of p-aminobenzoic acid by, 193
of niacin by, 186, 263
of thiamine by, 261
- Niacin, 165, 183, 226
absorption by microbial cells, 187
antagonism to adrenaline, 78
biochemical activity in plants, 263, 264, 268
biosynthesis of, 263
in blood, 118, 120, 154
cheilosis and, 144
content of microorganisms, 184
cozymase and, 263, 264
deficiency,
manifestations in roots, 236
skin and, 140
tongue and, 142
visual acuity and, 122
distribution in plants, 242, 243, 245, 246, 247
erythropoiesis and, 149
as growth factor for intact plants, 255
for roots, 228-230, 234 ff., 268, 269
inactivation of, 188
light and synthesis of, in germinating seedlings, 252, 253, 269
nervous system and, 147

- requirements for, 116
 - of hamster, 63
 - of rats, 58, 59
 - tryptophan and, 59, 79
- site of synthesis in plants, 269
- synthesis by bacteria, 185, 186
- therapeutic application of, 78, 79
- toxicity of, 77
- triphosphopyridine nucleotide and, 263, 264
- vasodilator action of, 70, 78
 - adrenaline and, 78
- Niacinamide,
 - root-growth activity of, 235
 - therapeutic effect in psychosis of pellagra, 71, 73
 - urinary excretion of, 235
- Niacinamide methiodide,
 - effect on root growth, 235
- Nicotinic acid,
 - root-growth activity of esters of, 235
- Nicotino-3-nitrile, effect on root growth, 235
- Nicotinuric acid,
 - root-growth activity of, 235
- Nutriture,
 - assessment of human, 101-162
 - methods used in, 156
 - cardiovascular function and, 128
 - definition of, 102
 - effect on hair, 138
 - nails, 138
 - skin, 138
 - endurance and, 126
 - growth rate and, 129 ff.
 - ischemic pain and, 126
 - neonatal mortality and, 132
 - normal, 112
 - physical fitness and, 126
 - pregnancy and, 131
 - pulmonary tuberculosis and, 131
 - respiratory function and, 128

O

- Oat, vitamins B in, 242, 245
 - Orchids, vitamin requirements of, 259
 - Oxidation-reduction enzyme systems,
 - role of pteroylglutamic acid in, 7
 - Oxybiotin, growth activity for microorganisms, 191
 - Oxypteroic acid, 17
 - Oxypteroylglutamic acid, 17
- P**
- Pantoic acid, requirements for, 265
 - Pantothenic acid, 164, 165, 181, 226, 265
 - biochemical role of, 266
 - biosynthesis from precursors, 183
 - coenzyme nature of, 181, 183, 266
 - content of microorganisms, 182
 - deficiency,
 - bacterial infection and, 81
 - eyes and, 144
 - hair and, 139
 - skin and, 139
 - distribution in plants, 242-245
 - effect of light on synthesis of, in germinating seedlings, 252, 253, 269
 - growth activity for plants, 259, 269, 270
 - for *Saccharomyces cerevisiae*, 181
 - requirement of hamster, 63
 - for moieties of, 265
 - of rats, 58
 - urinary excretion and, 74
 - role in human nutrition, 81
 - site of synthesis in plants, 248, 252, 269
 - toxicity of, 81
 - transport through plant, 248, 249, 269
 - Pantoyl lactone, biosynthesis of pantothenic acid from, 183
 - Pea, vitamin B content of, 245, 246
 - Pellagra,
 - niacinamide and, 71, 73
 - tongue in, 141
 - Penicillium chrysogenum*, 266
 - synthesis of riboflavin by, 182
 - Phosphatase,
 - effect of diet on serum, 120
 - Phosphorus,
 - connective tissues and, 148
 - teeth and, 143
 - Photosynthesis,
 - effect of vitamin K on, 37
 - Phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone),

- derivatives,
 - antagonism to menadione, 40, 41
 - to vitamin K₁, 40, 41
 - effect on algae, 37, 38
 - source of, 207
 - toxic effects of, 92
 - toxicity of, 92
- Phycomyces blakesleeana*, 261, 262
 - biosynthesis of biotin by, 266
 - of β -carotene by, 209, 213
 - of thiamine by, 261
- Phytin, 196
- β -Picoline. effect on root growth, 235
- Picolinic acid, effect on root growth, 235
- Pimelic acid, biosynthesis of biotin and, 191, 266
- Pituitary, anterior,
 - effect of sodium chloride on, 305
- Plant hormones, see also Auxins
 - vitamins B as, 225-270
- Plants,
 - biochemical activity of vitamins B in, 260-267
 - biosynthesis of thiamine in, 260-262
 - carboxylase in, 261
 - distribution of purines in, 249, 250
 - of vitamins B in, 225, 241-247
 - enzymes in, 261, 263
 - metabolism of purines in, 267
 - of vitamins B in, 260-267
 - transport of vitamins B through, 247-254
- Plasmodium lophurae*
 - biotin deficiency and resistance to, 83
- Potassium,
 - agents influencing distribution of, 283
 - antagonism of sodium to, 302, 303, 317, 318
 - content of muscle, 148, 282
 - effect of adrenalectomy on distribution of, 279, 280, 285
 - on excretion of, 288
 - of adrenocortical hormones on metabolism of, 312-321
 - retention of, 281, 307-312
- Pregnancy,
 - ascorbic acid and, 143
 - nutrition and, 131
- Procaine,
 - antagonism to vitamin K, 42
- Progesterone, effect on retention of chloride, 313
 - of sodium, 313
- Proteins,
 - in blood, 118, 154
 - effect of sodium chloride on metabolism of, following adrenalectomy, 296-298
 - in tissues, 118
- Proteus morganii*, biosynthesis of coenzyme A by, 266
- Prothrombin,
 - vitamin K and formation of, 33, 34
- Pseudomonas fluorescens*,
 - biotin content of, 189
- Pseudopyridoxine, 194
- Pterotic acid ([4-{(2-amino-4-hydroxy-6-pteridyl) methyl}-amino]-benzoic acid), 14
 - activity of, 18, 19, 201
 - antagonism of pteroylaspartic acid to, 16
- Pteroylaspartic acid,
 - antagonism to pteroylglutamic acid and its homologues, 16, 17
- Pteroyldiglutamic acid, synthesis of isomers of, 15
- Pteroylglutamic acid, see also Folic acid,
 - Liver *L. casei* factor, 64, 83, 198
 - antagonism of sulfonamides to, 18
 - deficiency syndrome, 17
 - effect on kidneys, 84
 - erythropoiesis and, 149
 - hematopoietic activity of, 84
 - identity with liver *L. casei* factor, 14
 - pharmacologic properties of, 84
 - requirements of rats, 58
 - structure of, 11, 22, 198
 - toxic effects of, 75
 - toxicity of, 84
- Pteroylglutamic acid compounds, see also under name of individual substances
 - activity of, 3, 4, 18-22
 - structure and, 22
 - antagonists of, 16-18
 - biosynthesis of, 18
 - chemistry of, 1-15
 - determination of, 21
 - synthesis of, 15, 16

- Pteroyl- α , γ -glutamyl diglutamic acid,**
 activity of, 19
 synthesis of, 16
- Pteroyl- γ -glutamyl glutamic acid,**
 activity of, 19
 antagonism of pteroylaspartic acid to, 16
- Pteroyl- γ -glutamyl- γ -glutamyl glutamic acid,** see also Pteroyltriglutamic acid
 activity of, 19
 antagonism of pteroylaspartic acid to, 16
 identity with fermentation *L. casei* factor, 15
 synthesis of, 15
- Pteroylhexaglutamyl glutamic acid,** see also Vitamin B₉ conjugate
 activity of, 19
- Pteroyltriglutamic acid,** identity with fermentation *L. casei* factor, 14, 15
 synthesis of isomers of, 15, 16
- Purines,** 226
 biosynthesis of, 267
 as leaf-growth factors, 240, 241
 metabolism of, in plants, 267
 occurrence in leaves, 249
 transport through plants, 249
- Pyrazine-3-carboxylic acid,** effect on root growth, 235
- Pyrazine-2,3-dicarboxylic acid,** effect on root growth, 235
- Pyridoxal,** as bacterial growth factor, 264
- Pyridoxal phosphate,** 194
 coenzyme nature of, 194, 264, 265
 synthesis by microorganisms, 195, 196
- Pyridoxamine,** as bacterial growth factor, 264
 biological conversion to codecarboxylase, 195, 196
 synthesis by microorganisms, 195
- Pyridoxine,** 164, 194, 226
 antagonism to atabrine, 71
 to quinine, 71
 biochemical activity in plants, 265
 content of microorganisms, 195
 deficiency
 acrodynia and, 139
 erythropoiesis and, 149
 manifestations in roots, 236
 skin and, 139
 distribution in plants, 243, 244, 245
 effect of light on synthesis of, in germinating seeds, 252, 253, 269
 on vegetal embryos, 269
 growth activity for plants, 255, 259, 260, 268
 immunity and, 80
 requirement of hamster, 63
 of rats, 58
 role of, in human nutrition, 80
 as root-growth factor, 228-230, 268, 269
 site of synthesis in plants, 248, 249, 252, 269
 sources of, 194
 synthesis by microorganisms, 195
 therapeutic application of, 71, 80, 81
 toxic effects of, 70
 toxicity of, 80
 transport through plant, 248, 249, 269
- Pyriethamine,**
 antagonism to thiamine, 169
 effect on microbial thiamine synthesis, 169

Q

- N-(4-[(4-Quinazoline)amino]benzoyl)-glutamic acid,**
 folic acid activity of, 17
- Quinine,** antagonism of pyridoxine to, 71
- Quinolinic acid,** effect on root growth, 235
- Quinones,** antibiotic effects of, 36

R

- Radiation sickness,** therapeutic effect of pyridoxine in, 71, 80
 of thiamine in, 77
- Rat, white,**
 choline requirement of, 58
 intestinal vitamin K synthesis by, 64
 vitamin B requirements of, 58
- Rhizopterin** (p-[N-(2-amino-4-hydroxypyrimido [4,5-b]pyrazin-6-ylmethyl)-formamido] benzoic acid, SLR factor), 201, 202
 absorption spectra of, 5, 12
 biological activity, 4, 5, 18, 19
 degradation of, 11-13

- empirical formula, 5
isolation of, 4, 5
molecular weight, 5
nature of, 5
solubility, 5
structure of, 12, 13
synthesis by *Rhizopus nigricans*, 5, 201
- Rhizopus nigricans*,
as source of rhizopterin, 5, 201
- Riboflavin, 165, 226
antagonism to p-dimethylaminoazo-
benzene, 80
biochemical activity in plants, 262, 263
in blood, 118, 120, 154
in cereal seeds, 247
content of microorganisms, 174
deficiency,
manifestations of, 121, 122, 130,
140, 142, 144-146
distribution in plants, 243-246
effect of light on synthesis of,
in germinating seeds, 251-253, 269
erythropoiesis and, 149
flavoproteins and, 262
glands and, 147
growth activity for plants, 259
muscular endurance and, 128
nervous system and, 147
requirement of hamster, 63
of rats, 58
site of synthesis in plants, 249, 252,
269, 270
stability of, 177, 178
therapeutic application of, 79, 80
toxic effects of, 75
toxicity of, 79
transport through plant, 248-250
in tumor tissue, 80
urinary excretion of, 121, 154
- Rickets,
diagnosis, 148
- Roots,
effect of auxin on growth of, 259
combined application of auxin and
thiamine on, 259, 260
glycine as growth factor for, 229, 230
manifestations of vitamin B deficiency
in, 236
synthesis of vitamins B by, 237, 239,
250, 260
- vitamins B as growth factors for, 226-
237
- Rutin,
anaphylaxis and, 89
histamine shock and, 89
toxicity of, 88
- S
- Saccharomyces carlsbergensis*,
as source of ergosterol, 206
- Saccharomyces cerevisiae*,
p-aminobenzoic acid content of, 193
synthesis by, 192-194
vitamin B content of, 166, 174, 182,
184, 185, 199
synthesis by, 169, 178, 183, 196
- Salicylates,
hemorrhagic activity of, 41
synergism of p-aminobenzoic acid with,
82
- Seeds, behavior of vitamins B during
germination of, 251-254
- Serum, blood,
effect of vitamin K on agglutinating
power of, 44
- Sigmodon hispidus hispidus*, see Cotton
rat
- Sigmodon hispidus littoralis*, see Cotton rat
- Skin,
effect of fatty acid deficiency on, 139
of malnutrition on, 140
of vitamin deficiencies on, 138 ff.
- Sodium,
antagonism to potassium, 302, 303,
317, 318
content of muscle, 282
deficiency symptoms, 144
effect of adrenalectomy on distribution
of, 279, 280-284
on excretion of, 287, 288
of adrenocortical hormones on metab-
olism of, 312-321
retention of, 281
adrenocortical extract and, 312, 313
transfer through intestinal wall, 290-
292
- Sodium chloride,
adrenalectomy and physiological ef-
fects of, 295-305

- effect on anterior pituitary, 305
 - on estrus cycle, 305
 - on renal function, 320
- loss of, in adrenal insufficiency, 287, 288, 306, 307
- Spermatogenesis, amino acids and, 149
- Spinach, vitamin B content of, 243
- Sprue, therapeutic effect of
 - pteroylglutamic acid in, 85
- Squash, vitamin B content of, 246
- Staphylococcus aureus*,
 - synthesis of pteroylglutamic acid by, 18
- Stem-growth factors, 241
- Streptococcus faecalis* R, 7
 - growth activity of liver *L. casei* factor for, 3, 18
 - of pteric acid for, 18
 - of vitamin B₆ conjugate for, 2
- Streptococcus lactis* R, 5
 - growth activity of rhizopterin for, 4, 5
- Succinic acid, as growth factor for vegetal embryos, 238
- Sulfadiazine, vitamin K deficiency and, 2
- Sulfaguanidine, effect on biosynthesis of niacin, 187
- Sulfaquinoxaline (2-sulfanilamidoquin-oxaline)
 - hypoprothrombinemic activity of, 42
 - menadione and, 42, 71, 93
 - vitamin K₁ and, 42, 93
- Sulfonamides,
 - antagonism to p-aminobenzoic acid, 71, 193
 - to pteroylglutamic acid, 18, 84
 - effect on biosynthesis of biotin, 83
 - of folic acid, 202
 - of thiamine, 74
- Synkayvite (tetrasodium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid), see also Vitamin K
 - daily requirement of the newborn, 43
 - effect on mitosis, 38
 - hyperprothrombinemia and, 34
- phosphorus and, 143
- vitamins and, 143
- Testosterone propionate,
 - effect on retention of chloride, 313
 - of sodium, 313
- Tetrahydrovitamin B₆, 6
 - absorption spectrum of, 6, 7
- Theobromine, distribution in plants, 249, 250
- Thiamine, 165, 226
 - absorption by microorganisms, 171-173
 - analogues,
 - root-growth activity of, 231-234
 - chemical structure and, 234
 - anorexia and, 127
 - beriberi and, 149
 - biochemical activity in plants, 262, 268
 - biosynthesis of,
 - effect of antibiotics on, 74
 - of dietary fat on, 74
 - of sulfonamides on, 74
 - by microorganisms, 169-171
 - in plants, 260-262
 - from precursors, 170, 171
 - in blood, 119, 120, 154
 - cardiovascular function and, 128
 - in cereal seeds, 247
 - content of microorganisms, 166, 167
 - deficiency,
 - manifestations in roots, 236
 - visual acuity and, 121
 - distribution in plants, 242-246
 - effect on cuttings, 259
 - on learning in children, 129
 - of light on synthesis of, in germinating seeds, 251-253, 269
 - on niacin biosynthesis, 186
 - as growth factor for intact plants, 254, 255, 258
 - for roots, 228-234, 259, 260, 268, 269
 - for vegetal embryos, 269
 - inactivation of, 173, 262
 - inhibitory effect on choline esterase formation, 77
 - ischemic pain and, 128
 - mental depression and, 127
 - muscles and, 128, 148, 149

T

- Tachysterol, 90
- Teeth, calcium and, 143
- fluorine and, 143, 144

- nervous system and, 147, 148
 - pyrithiamine and, 169
 - requirements, 116, 128
 - of hamster, 63
 - of rats, 58
 - urinary excretion and, 74
 - site of synthesis in plants, 237, 239, 248, 251, 252, 260, 269
 - therapeutic application of, 71, 77
 - toxic effects of, 70, 76
 - manganese and, 76
 - mode of administration and, 75, 76
 - toxicity of, 75
 - transport through the plant, 248, 250, 251, 253, 254, 269
 - urinary excretion of, 74, 121, 154
 - Thiamine esters, occurrence in micro-organisms, 172, 173
 - Thiamine pyrophosphate, coenzyme nature of, 261
 - Thiazole-5-carboxylic acid,
 - effect on root growth, 235
 - Thyroid gland,
 - effect of fluorine on, 147
 - of iodine on, 147
 - Tissues, chloride-containing
 - cells in, 282
 - composition of, 117
 - connective,
 - minerals and, 148
 - vitamins and, 148
 - α -Tocopherol, see also Vitamin E
 - therapeutic effect in habitual abortion, 91
 - in neuromuscular disorders, 91, 92
 - α -Tocopherolquinone,
 - antagonism to vitamin E, 40
 - vitamin K and, 40
 - Tocopherols, antioxidant activity of, 91
 - antisterility activity of, 91
 - synergism with carotene, 91
 - with vitamin A, 91
 - Tomato, vitamin B content of, 242, 244, 246
 - Torula utilis*, inositol synthesis by, 197
 - Toxisterol, 89
 - Triphosphopyridine nucleotide,
 - enzymes and, 263, 264
 - niacin and, 263, 264
 - Trypanosoma lewisi*, biotin deficiency
 - and resistance to, 83
 - Tryptophan, biological conversion to
 - niacin, 59, 186
 - effect on niacin requirement, 79
 - Tumors,
 - biotin content of, 83
 - effect of pteroylglutamic acid compounds on, 85
 - riboflavin content of, 80
- U
- Uric acid, as metabolite of xanthine, 267
 - occurrence in plant tissue, 267
 - Urine,
 - composition, 120
- V
- Vision,
 - effect of vitamins on, 121, 122
 - Vitamin A,
 - antagonism of vitamin K to, 89
 - to vitamin D, 90
 - in blood, 152, 154
 - connective tissues and, 148
 - deficiency,
 - hair and, 139
 - skin and, 138, 139
 - Eales' disease and, 111
 - effect of diet on, in blood, 119, 120
 - on growth, 130
 - on intestinal vitamin K synthesis, 41
 - eyes and, 144, 146, 147
 - glands and, 147
 - metabolism, 123
 - relation between dietary, and dicumarol toxicity, 40
 - respiratory system and, 149
 - rod threshold of dark-adapted eye and, 122-124
 - teeth and, 143
 - therapeutic effect in follicular hyperkeratosis, 90
 - in night blindness, 90
 - toxic effects of, 89
 - Vitamin B₁₀,
 - nature of, 20
 - Vitamin B₁₁, nature of, 20

- Vitamin B complex,
 chronaxie and, 125
 deficiency,
 effect of thiamine on, 72
 on mental capacity, 127, 129
 on physical fitness, 127, 128
 psychiatric manifestations of, 72
 visual disturbances and, 122
 effect of diet on, in blood, 120
 muscular strength and, 128
 urinary excretion of, 121
- Vitamin B₆, see also Folic acid, Liver L.
casei factor, Pteroylglutamic acid
 activity of, 2
 conversion to dehydrovitamin B₆, 6
 to tetrahydrovitamin B₆, 6
 degradation of, 5-7
 isolation of, 1, 2
- Vitamin B₆ conjugase (pteroylglutamic
 acid conjugase), 20, 21
 inactivation of, 21
 sources of, 20, 21
- Vitamin B₆ conjugate, 1
 absorption spectrum of, 2, 3
 constitution of, 7
 degradation of, 7
 growth activity for *L. casei*, 2
 for *Streptococcus faecalis*, 2
 isolation of, 2, 3
- Vitamin C,
 requirement of cotton rat, 60
 of hamster, 63, 64
- Vitamin D,
 antagonism of vitamin A to, 90
 connective tissues and, 148
 nails and, 141
 teeth and, 143
 therapeutic effect in infantile rickets,
 91
 in rheumatic arthritis, 71
 toxic effects of, 90
 toxicity, 75, 90
 source and, 90
- Vitamin D₂ (calciferol), 90
 toxic effects of, 90
- Vitamin D₃ (activated 7-dehydrosterol),
 90
 source of, 90
 toxic effects of, 90
- Vitamin E, see also α -Tocopherol, 91, 92
 abortion and, 148
 chronaxie and, 125
 heart disease and, 92
 muscles and, 148
 requirement of hamster, 63, 64
- Vitamin K, see also Menadione, 28-48
 acetylcholine synthesis and, 93, 207,
 208
 antagonism to vitamin A, 89
 antagonists of, 39-42
 deficiency,
 hemorrhage due to, 35
 manifestations of, 140
 mineral oil and, 41
 in pulmonary tuberculosis, 44
 sulfa drugs and, 41
 in ulcerative colitis, 44
 dental caries and, 46, 47
 determination of, 29-33
 effect on agglutinating power of serum, 44
 on photosynthesis, 37
 proteolytic enzymes, 35
 serum antithrombin, 35
 intestinal synthesis, 64
 dihydroxystearic acid and, 41
 massive vitamin A doses and, 41
 liver function tests with, 45, 72
 prothrombin formation and, 33, 34
 requirements of hamster, 63, 64
 of the newborn, 43
 sources of, 207
 therapeutic application of, 43-46
- Vitamin K₁ (3-phytylmenadione),
 antagonism to dicumarol, 40, 71
 to phthiocol derivatives, 40, 41, 93
 biological conversion to menadione, 34
 determination of, 30-32
 effect on acetylcholine synthesis, 93
 on blood pressure, 92
 therapeutic effect in hypoprothrom-
 binemia, 93
 toxicity of, 92
- Vitamin K₂ (3-difarnesylmenadione),
 as bacterial growth factor, 208
 bacterial synthesis of, 208
 biological conversion to menadione, 34
 determination of, 30
 sources of, 29

- Vitamin K₁,
 effect on blood coagulation, 29
 solubility of, 29
 source of, 29
- Vitamin K₂, see 4-Amino-2-Methyl-1-naphthol hydrochloride
- Vitamin K compounds,
 bacteriostatic effect of, 36
 dental caries and, 93
 growth activity of, 37
 therapeutic effect in hypoprothrombinemia, 93
 toxic effects of, 92
- Vitamin P (citrin),
 capillary fragility and, 88, 125
 sources of, 88
 toxicity of, 88
- Vitamins,
 in blood, 118 ff.
 distribution in microorganisms, 164-214
 intestinal synthesis of, 165
 mode of administration and toxic effects of, 74-76
 as pharmacological agents, 69-93
 specificity of, 72, 73
 synthesis by microorganisms, 164-214
 urinary excretion and requirement for, 74
 water-soluble, in leucocytes, 117
- Vitamins B,
 coenzyme nature of, 213
 distribution in the plant, 241-247
 in fermentation residues, 210-212
 as plant hormones, 225-270
 site of synthesis in plants, 248-251
- Vitalol, see Menadione bisulfite

W

- Water,
 distribution of, 284
 effect of adrenal cortex on metabolism of, 284-287, 289-291, 293
 of estrogens on metabolism of, 313
 transfer through intestinal wall, 290-292
- Wounds,
 ascorbic acid and healing of, 87, 148

X

- Xanthine,
 biological conversion to uric acid, 267
 distribution in plants, 249, 250
 as metabolite of guanine, 267
 of hypoxanthine, 267
- Xanthopterin, 202
- Xanthurenic acid, effect of desoxypyridoxine on urinary excretion of, 71

Y

- Yeasts,
 absorption of ascorbic acid by, 203
 of niacin by, 187
 of thiamine by, 171
 preparation of thiamine enriched, 172
 as source of p-aminobenzoic acid, 192, 193
 of ergosterol, 206, 207
 of folic acid, 198, 200
 of inositol, 197
 of pyridoxine, 194
 of thiamine, 169-171
 thiamine content of, 166

Z

- Zinc,
 deficiency symptoms, 139, 144

